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Effects of fermented vermicompost on the physiological and biochemical responses of plants subjected to abiotic stress

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Project Title:

VermiHerb Shield – Production of Organic Liquid Biostimulants for Hydroponic Agriculture

VERMIHERB SHIELD

SHIELD EXTRACTION

Presented by: SABOUR Bochra

Before the jury composed of:

Name & Surname	Grade	Institution	Role
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With a heart overflowing with love, I dedicate this milestone to all of you.

BOCHRA

Effects of fermented vermicompost on the physiological and biochemical responses of plants subjected to abiotic stress

Abstract

Tomato holds a crucial position on both economic and nutritional scales worldwide. However, its production is severely limited by various biotic and abiotic stresses, particularly salt stress.

To cope with these constraints, plants have developed adaptive strategies enabling them to maintain their homeostasis and viability under unfavourable conditions. To strengthen these defence mechanisms, the use of natural products, commonly referred to as biostimulants, has been widely developed. Among modern bio-ecological approaches, vermicompost-derived fermentations represent a promising technology, valorising agricultural residues while contributing to the sustainable management of plant health.

The general objective of this study is to evaluate the effect of three types of fermentations, namely a vermicompost tea-based fermentation, a fermentation of aromatic plants (Eucalyptus, Bermuda grass, nettle, rosemary, fleabane, and wild mustard), and a combined fermentation of vermicompost tea and aromatic plants. Each of these fermentations is applied as a liquid extract at two concentrations (5 PERCENT and 10 PERCENT) on various morphological parameters, photosynthetic pigments (chlorophylls a, b, and total), phenolic and flavonoid compounds, as well as the accumulation of soluble solutes (proline and total sugars) in tomato seedlings (*Solanum lycopersicum* L., 1753) subjected to salt stress.

The results obtained demonstrate that the application of the different biostimulants exerted a beneficial effect on all morphological parameters of tomato, compared to the control. In particular, the growth parameters revealed a notable improvement in vegetative growth in treated plants subjected to salt stress, relative to the control plants. Furthermore, the analysis of physio-biochemical leaf parameters highlighted a stimulating effect of the biostimulants on chlorophyll, polyphenol, and flavonoid contents.

Among the treatments tested, the biostimulant based on vermicompost combined with fermented aromatic plants proved to be the most effective. It was followed by fermented vermicompost, and then by plant-based fermented biostimulants. In addition, the evaluation of microbial activity revealed a higher microbial load in biostimulants containing vermicompost, which could explain, at least in part, their superior efficacy on the growth and physiological responses of tomato seedlings.

Keywords: Biostimulants; fermentation; morphological parameters; physio-biochemical parameters; salt stress; vermicompost.

Effets du lombricompost fermenté sur les réponses physiologiques et biochimiques de plantes soumises à un stress abiotique

Résumé

La tomate occupe une position cruciale sur les plans économique et nutritionnel à l'échelle mondiale. Cependant, sa production est fortement limitée par divers stress biotiques et abiotiques, notamment le stress salin. Pour faire face à ces contraintes, les plantes ont développé des stratégies adaptatives leur permettant de maintenir leur homéostasie et leur viabilité dans des conditions défavorables. Afin de renforcer ces mécanismes de défense, l'utilisation de produits naturels, communément appelés biostimulants, s'est largement développée. Parmi les approches bioécologiques modernes, les fermentations issues du vermicompost représentent une technologie prometteuse, valorisant les résidus agricoles tout en contribuant à une gestion durable de la santé des plantes. L'objectif général de cette étude est d'évaluer l'effet de trois types de fermentations, à savoir une fermentation à base de thé de vermicompost, une fermentation de plantes aromatiques (Eucalyptus, chiendent pied-de-poule, ortie, romarin, inule et moutarde des champs), et une fermentation associant le thé de vermicompost et les plantes aromatiques. Chacune de ces fermentations est appliquée sous forme de purin à deux concentrations (5 PURCENT et 10 POURCENT), sur divers paramètres de morphologique, les pigments photosynthétiques (chlorophylles a, b et totales), les composés phénoliques et flavonoïdiques, ainsi que l'accumulation de solutés solubles (proline et sucres totaux) chez des plants de tomate (Solanum lycopersicum L., 1753) soumis à un stress salin.

Les résultats obtenus montrent que l'application des différents biostimulants a exercé un effet bénéfique sur l'ensemble des paramètres morphologiques de la tomate, comparativement au témoin. En particulier, les paramètres de croissance ont révélé une amélioration notable de la croissance végétative chez les plants traités et soumis au stress salin, par rapport aux plants témoins. Par ailleurs, l'analyse des paramètres physio-biochimiques foliaires a mis en évidence un effet stimulant des biostimulants sur les teneurs en chlorophylles, en polyphénols et en flavonoïdes. Parmi les traitements testés, le biostimulant à base de vermicompost associé à des plantes aromatiques fermentées s'est montré le plus performant. Il est suivi par le vermicompost fermenté, puis par les biostimulants à base de plantes fermentées. En outre, l'évaluation de l'activité microbienne a révélé une charge microbienne plus importante dans les biostimulants contenant du vermicompost, ce qui pourrait expliquer, au moins en partie, leur efficacité supérieure sur la croissance et les réponses physiologiques des plants de tomate.

Mots-clés : Biostimulants; fermentation; parèmètres morphologiques ; parèmètres physio-biochimique; stress salin; vermicompost.

تأثيرات لومبيركوموبست المخمر على الاستجابات الفسيولوجية والكيميائية الحيوية لنباتات تحت إجهاد لاحيائي

الملخص

تحتل الطماطم مكانة حاسمة على الصعيدين الاقتصادي والتغذوي على مستوى العالم. ومع ذلك، فإن إنتاجها يُحَد بشكل كبير من العديد من الضغوطات الحيوية وغير الحيوية، ولا سيما الإجهاد الملحي. لمواجهة هذه الضغوطات، طورت النباتات استراتيجيات تكيفية تسمح لها بالحفاظ على توازنها الداخلي وقدرتها على البقاء في الظروف غير الملائمة. ولتعزيز هذه الأليات الدفاع، انتشر استخدام المنتجات الطبيعية، المعروفة باسم المحفزات الحيوية، على نطاق واسع. ومن بين المناهج البيوتكنولوجية الحديثة، تمثل التخمرات المستخلصة من لوميركوموبست تقنية واعدة، حيث تساهم في تثمين المخلفات الزراعية مع المساهمة في إدارة مستدامة لصحة النبات.

الهدف العام لهذه الدراسة هو تقييم تأثير ثلاثة أنواع من التخمرات، وهي: تخمير قائم على شاي لوميركوموبست، وتخمير نباتات طبية وعطرية (الأوكالبتوس، خرس النمر، القراص، إكليل الجبل، الإبنول، والخردل البري)، وتخمير يجمع بين شاي لوميركوموبست والنباتات الطبية والعطرية، كل تخمير بطُبق على شكل سائل مخمر (بورين) بتركيزين (%1 و10%)، على مختلف المعايير المورفولوجية، والأصباغ التركيبية الضوئية (الكلوروفيل أ، ب، والإجمالي)، والمركبات الفينولية والفلافونويدية، بالإضافة إلى تراكم المواد الذائبة (البورولين والسكريات الكلية) في نباتات الطماطم(*Solanum lycopersicum* L., 1753) المعرضة للإجهاد الملحي.

أظهرت النتائج المتحصل عليها أن تطبيق المحفزات الحيوية المختلفة كان له تأثير إيجابي على جميع المعايير المورفولوجية للطماطم، مقارنة بالشاهد. وبشكل خاص، كشفت معايير النمو عن تحسن ملحوظ في النمو الخضري للنباتات المعاملة والمعرضة للإجهاد الملحي، مقارنة بالنباتات الشاهدة. بالإضافة إلى ذلك، أظهر تحليل المعايير الفسيولوجية-الكيميائية الحيوية للأوراق تأثيراً محفزًا للمحفزات الحيوية على محتوى الكلوروفيلات، ومتعدد الفينولات، والفلافونويدات. ومن بين المعاملات المختبرة، كان المحفز الحيوي القائم على لوميركوموبست المرتبط بنباتات طبية وعطرية مخمرة هو الأكثر فعالية، يليه لوميركوموبست المخمر، ثم المحفزات الحيوية القائمة على نباتات مخمرة.

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الكلمات المفتاحية: المحفزات الحيوية؛ التخمير؛ المعايير المورفولوجية؛ المعايير الفسيولوجية-الكيميائية الحيوية؛ الإجهاد الملحي؛ لوميركوموبست.

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General Introduction

Plants are exposed throughout their life cycle to various biotic stresses, such as pests, pathogenic microorganisms, and nematodes, as well as abiotic stresses, including thermal, water, and salt stresses, all of which affect plant growth and lead to variable yield losses [Munns and Tester, 2008]. Among the main abiotic stresses, salinity is one of the most severe constraints on global agricultural productivity. It currently affects more than 800 million hectares of cultivated land and continues to expand due to climate change, overexploitation of groundwater resources, and irrigation with saline water [Alamer et al., 2022].

The accumulation of salts in the soil, particularly sodium chloride (NaCl), induces a dual physiological constraint: an osmotic component that reduces water and nutrient uptake, and an ionic component that disrupts K^+/Na^+ homeostasis as well as enzymatic functions. These disturbances lead to an over-accumulation of reactive oxygen species (ROS), including the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•), resulting in lipid membrane peroxidation, protein oxidation, and damage to nucleic acids [Altaf et al., 2023; Hasanur Rahman et al., 2019; Munns and Tester, 2008].

In response to these constraints, plants mobilise a complex network of defence mechanisms, including osmotic adjustment through the accumulation of compatible solutes (proline, glycine betaine, and soluble sugars), the activation of enzymatic antioxidant systems [superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD)], as well as the reconfiguration of photosynthetic activity involving stomatal closure and down-regulation of chlorophyll biosynthesis [Altaf et al., 2023; Rivero et al., 2023]. The quantification of these parameters therefore represents a standard approach for assessing both stress severity and the effectiveness of applied protective treatments in plants [Alamer et al., 2022].

To enhance plant defence responses, natural products commonly referred to as biostimulants are widely used. These substances or microorganisms are applied to plants or their rhizosphere in order to improve growth, development, resistance to both abiotic and biotic stresses, as well as nutrient use efficiency. By optimising nutrient uptake and assimilation, biostimulants enable plants to become more robust and resilient, as highlighted by Calvo et al. [2014a].

Among the main biostimulants, vermicompost has attracted increasing scientific interest. It is produced through the joint action of earthworms and associated microorganisms on organic matter at ambient temperature, resulting in a humus-rich product characterised by high concentrations of humic and fulvic acids, plant-available macro- and micronutrients (N, P, K, Ca, Mg, Fe), phytohormones (indole-3-acetic acid, gibberellic acid, and cytokinins), as well as beneficial microorganisms [Arancon et al., 2004; Paneque et al., 2024].

It has been demonstrated that this type of product promotes plant growth, stimulates root development through hormonal mechanisms, and enhances antioxidant defence systems in crops subjected to abiotic stresses [Mosa et al., 2026; Rehman et al., 2023; Romano et al., 2019].

Several studies have shown that the fermentation of vermicompost tea in the presence of aromatic plants enriches the final product with bioactive compounds such as phenolic compounds, flavonoids, as well as various secondary metabolites with antioxidant and antimicrobial properties. This biotransformation process therefore represents a promising avenue of innovation in the field of biostimulants, particularly for the development of more effective natural formulations adapted to abiotic stresses [Colla et al., 2015; Sadak et al., 2025].

This work is part of sustainable (integrated) agriculture, a durable approach aimed at improving and ensuring crop productivity while preserving biodiversity and promoting environmentally friendly agricultural practices.

The main objective of this study is to evaluate and compare the effects of three types of fermentations: a vermicompost tea-based fermentation, an aromatic plant fermentation, and a combined fermentation of vermicompost tea and aromatic plants. Each of these fermentations is applied as a liquid extract (plant extract) at two concentrations (5% and 10%).

This study focuses on the analysis of various growth parameters, as well as photosynthetic pigments (chlorophyll a, b, and total chlorophylls, and carotenoids), phenolic and flavonoid compounds, and the accumulation of soluble osmolytes (proline and total sugars) in tomato plants (*Solanum lycopersicum*) subjected to salinity and water stress.

Chapter 1

Bibliographic Review

1.1 Salt stress in plants

Introduction

Throughout their life cycle, plants are constantly exposed to environmental constraints that induce complex physiological and biochemical changes, including osmotic imbalance, oxidative damage, and disruption of metabolic pathways. These alterations progressively affect plant growth, development, and productivity, potentially compromising their survival. These constraints are collectively referred to as plant stress Shavrukov et al. [2012].

In biology, stress is described as a significant deviation from normal environmental conditions that has a major impact on an organism and may result in injury. Thus, biological stress is defined as "any unusual environmental factor imposing a potentially injurious strain" Stolker [2010]. Two types of stress are distinguished: **biotic** and **abiotic** stress. Biotic stress results from damage caused by living organisms such as insects, pathogens, and weeds. Abiotic stress, on the other hand, refers to non-living environmental factors that can negatively affect plants, such as drought, cold, frost, high temperatures, high light intensity, and salinity. Abiotic stresses can significantly impair plant growth and development by causing an excess or deficiency of physical or chemical components. Because plants are sessile organisms, they rely entirely on the environmental conditions of their growing location.

These stresses pose major challenges and constitute significant limitations to crop productivity, and may even lead to plant death Stolker [2010]. It is therefore increasingly important to develop crop varieties with tolerance to multiple stresses in order to mitigate the impacts of environmental changes and meet the growing demands of an expanding global population, especially since various abiotic stresses often occur simultaneously under field conditions He et al. [2018].

1.1.1 Definition of Salt Stress

Salinity stress is a complex mechanism resulting from the excessive accumulation of salts within plant cells to levels that can alter the plant's physiological and biochemical processes as well as its morphological characteristics Abbasi et al. [2016]. It can lead to membrane disorganization, the production of toxic metabolites, inhibition of photosynthesis, generation of reactive oxygen species (ROS), and reduced nutrient acquisition, which may ultimately result in cell death or even the death of the entire plant Abbasi et al. [2016]. Salinity stress also reduces the ability of roots to absorb water and simultaneously induces ionic toxicity, osmotic stress, and oxidative stress Rahman et al. [2019].

Salinity stress is one of the most detrimental environmental stresses affecting plants, as it limits

agricultural productivity by negatively impacting seed germination, plant growth, development, crop yield, and vigor. Moreover, it significantly reduces food production by decreasing crop performance and limiting the availability of arable land. According to the United Nations Environment Program, salt stress affects nearly 20% of agricultural land in the world and approximately 50% of cultivated land worldwide Hassan [2024].

Salinity buildup is also a major constraint in hydroponic agriculture, particularly in recirculating systems. In these systems, limited leaching, continuous nutrient recycling, and nutritional imbalances lead to an increase in ionic concentration, resulting in higher electrical conductivity of the nutrient solution Renganathan et al. [2025]. Despite the precise control of nutrient solutions and environmental parameters in hydroponics, any variation can induce stress, particularly salinity stress, leading to reduced yields in crops such as lettuce, *cai xin* (*Brassica rapa*), and spinach Lee et al. [2025]. Furthermore, hydroponics provides a reliable experimental system for conducting reproducible stress studies by eliminating soil variability, thereby facilitating the investigation of plant stress tolerance mechanisms Shabala [2013].

1.1.2 Salinity Causes

Salinity can be divided into two main types based on its origin: **primary** and **secondary** salinity Hassan [2024]. Primary salinity arises from natural processes and sources such as salt marshes, salt lakes, tidal wetlands, and naturally occurring salt deposits. In contrast, secondary salinity is mainly driven by human activities, including urban development and agricultural practices under both irrigated and rainfed systems.

The factors contributing to salinity can be grouped as follows Hassan [2024]:

Primary salinity. Primary salinity is caused by natural processes, including rock weathering, the upward movement of shallow saline groundwater through capillary action, the deposition of salt-bearing sand transported by wind from marine environments, seawater intrusion in coastal areas, and poor or restricted drainage conditions.

Secondary salinity (due to human activity). Secondary salinity results from human activities such as irrigation practices lacking proper drainage systems, excessive use of fertilizers, discharge of industrial effluents, removal of natural vegetation cover, a high water table combined with the use of low-quality irrigation water, and inundating with salt-rich water.

1.1.3 The Effects of Salinity on Plants

Salt stress exerts its harmful effects through multiple mechanisms. Excessive accumulation of sodium (Na^+) and chloride (Cl^-) ions disrupts numerous intracellular metabolic processes and

impairs normal cellular functions. High salt concentrations induce osmotic stress, reducing the ability of roots to absorb water. Furthermore, elevated Na^+ levels disrupt cellular homeostasis and interfere with nutrient uptake and assimilation, while the combined effects of ion toxicity and osmotic stress lead to metabolic and oxidative imbalances. Salinity can reduce plant productivity and, under severe conditions, cause plant death Hassan [2024].

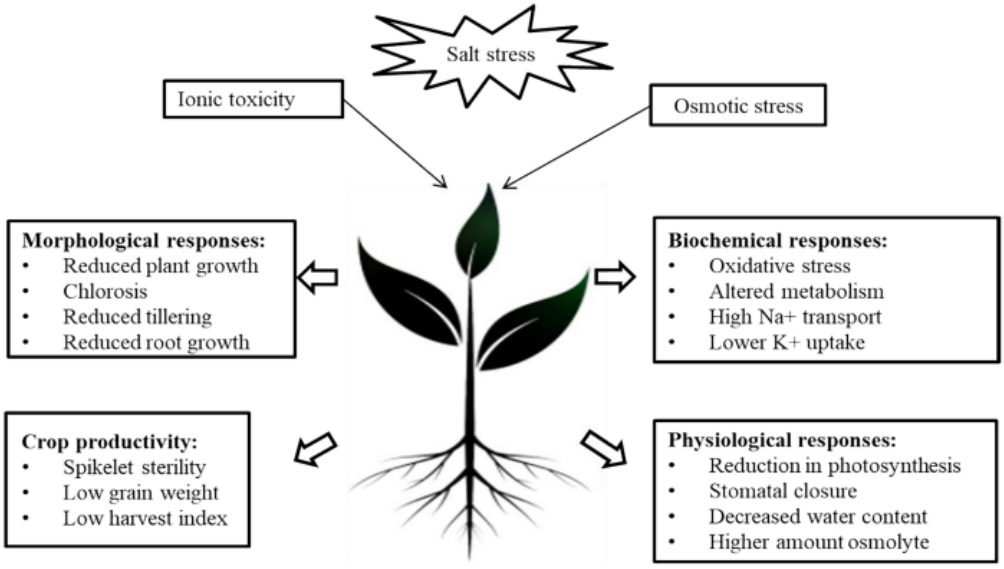


Figure 1.1: Impact of salinity stress on various physiological and morphological traits of plants. Ionic toxicity leads to morphological changes (reduced growth, chlorosis) while osmotic stress causes biochemical and physiological disturbances. Adapted from Singh et al. [2022].

Munns [1995] (Hassan [2024]) proposed a two-phase model to explain the effects of salinity stress in plants (Figure 1.2). The level of tolerance of plant species to salinity varies depending on the species and the intensity of the stress.

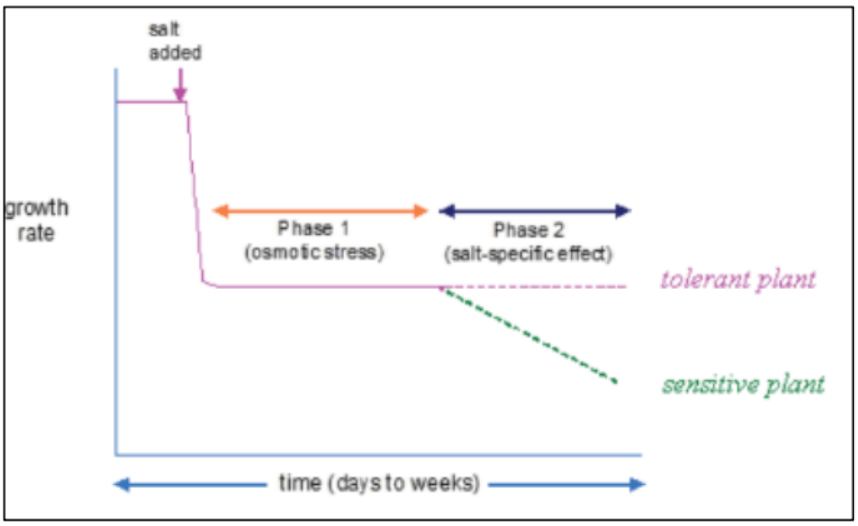


Figure 1.2: Scheme of the two-phase growth response to salinity, showing the osmotic phase (Phase 1) and the salt-specific ion-toxicity phase (Phase 2) for tolerant vs. sensitive plant genotypes. Adapted from Hassan [2024].

- **The osmotic phase (Phase 1)** occurs rapidly after salt exposure and is characterized by a reduced ability of roots to absorb water, leading to decreased shoot growth, stomatal closure, and reduced photosynthesis. It is also associated with disturbances in calcium (Ca^{2+}) transport and inhibition of essential mineral nutrient absorption such as potassium (K^{+}) and calcium (Ca^{2+}) Hassan [2024].
- **The ionic phase (Phase 2)** occurs later and corresponds to the progressive accumulation of sodium (Na^{+}) in the leaves, where it becomes toxic, particularly in older tissues. This accumulation strongly reduces photosynthetic capacity, thereby limiting the production of carbohydrates required for growth. Excess sodium also affects components of the photosynthetic system, including enzymes, chlorophylls, and carotenoids. This impairment of photosynthesis promotes oxidative stress through the production of reactive oxygen species (ROS) and may lead to leaf senescence and death Hassan [2024].

Plant responses to salinity stress also involve complex signalling mechanisms, particularly through abscisic acid (ABA) and calcium (Ca^{2+}), which regulate the expression of stress-tolerance genes, thereby contributing to plant protection and survival Hassan [2024].

1.1.4 Effects on Plant Growth and Development

Salinity stress affects all plant growth processes, from germination to vegetative and reproductive development. Its effect varies depending on salt concentration, plant species, variety, af-

fected organ, and developmental stage. The main symptoms include reduced vegetative growth, characterized by poor branching, decreased leaf expansion rate, a reduction in the number of nodes and leaves, and shortened stems. A decrease in both fresh and dry biomass of roots, stems, and leaves is commonly reported Oukara [2018]. Salt accumulation, particularly at high sodium concentrations, limits plant growth by increasing the osmotic pressure of the medium and inducing specific ion toxicity.

1.1.5 Effects on Photosynthesis

Photosynthesis is negatively affected by salinity stress through two main mechanisms. First, plants close their stomata under salt stress, which reduces the concentration of CO_2 inside the leaves and consequently limits carbon fixation. Second, salt stress results in various non-stomatal limitations, including damage to the photosynthetic membrane system, reduced CO_2 assimilation capacity, and degradation of photosynthetic pigments Sharma et al. [2023].

Salinity stress disrupts the functioning of both stomata and chloroplasts. Following stress, the accumulation of Na^+ and Cl^- ions in leaves, together with a reduction in potassium (K^+) levels, promotes stomatal closure, thereby restricting CO_2 entry and reducing photosynthetic activity. Furthermore, salinity decreases chlorophyll content and alters the ultrastructure of chloroplasts, particularly the thylakoid membranes Sharma et al. [2023].

1.1.6 Effects on Ion Homeostasis

Salinity stress disrupts plant ion homeostasis by altering the uptake, transport, and distribution of essential ions Govea et al. [2025]. Excessive NaCl concentrations inhibit the absorption and accumulation of several essential nutrient ions, particularly potassium (K^+), leading to nutrient imbalances. An increase in NaCl leads to elevated levels of sodium (Na^+) and chloride (Cl^-) ions within plant cells, accompanied by a decrease in calcium (Ca^{2+}), potassium (K^+), and magnesium (Mg^{2+}) concentrations Hassan [2024].

1.1.7 Osmotic Effects

The presence of high concentrations of soluble salts in the soil creates elevated osmotic pressure in the root environment, thereby reducing water availability for plants. This phenomenon is known as *physiological drought*. When osmotic adjustment is insufficient, water tends to leave the cells, leading to a loss of turgor pressure Hamdoud [2012]. Calcium also contributes to osmotic adjustment by regulating the intracellular K^+/Na^+ ratio Hamdoud [2012].

1.1.8 Effects on Reactive Oxygen Species (ROS)

Salt stress leads to an increase in the production of reactive oxygen species (ROS) in plant cells, including H_2O_2 (hydrogen peroxide), $O_2^{\bullet -}$ (superoxide anion), $^1O_2$ (singlet oxygen), and $OH^{\bullet}$ (hydroxyl radical). This overproduction results in oxidative stress, causing significant cellular damage such as lipid peroxidation, protein degradation, and DNA mutations Abbasi et al. [2016].

To cope with this stress, plants develop an antioxidant defence system composed of:

- **Non-enzymatic antioxidants**: tocopherols, carotenoids;
- **Enzymatic antioxidants**: superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), glutathione reductase (GR), and ascorbate peroxidase (APX).

Studies show that salt-tolerant varieties generally exhibit higher antioxidant enzyme activity, which confers greater resistance to oxidative stress Abbasi et al. [2016].

1.1.9 Effects on Energy Balance

Salinity stress disrupts the energy balance of plants by affecting the major metabolic processes involved in energy production and utilization. Under normal conditions, the efficiency of energy conversion into biomass is relatively high. However, under salinity stress, this efficiency decreases considerably due to the additional energy costs associated with salt adaptation and tolerance mechanisms Gharabi [2018].

1.1.10 Molecular Mechanisms of Plant Salt Stress Tolerance

Tolerance to salt stress in plants involves the regulation of multiple physiological and biochemical processes, including osmoregulation and osmotic adjustment, hormonal regulation, activation of the antioxidant defence system, and ion homeostasis. Different plants exhibit varying responses and mechanisms of adaptation Farooq et al. [2017].

1.1.11 Hormonal Regulation

Phytohormones are molecules that regulate plant growth and development. Under salt stress, the levels of abscisic acid (ABA) and ethylene are generally altered. In some species, exposure to salinity leads to stomatal closure, initially in response to locally produced ABA, followed by ABA synthesized in the roots and transported to the leaves. The increase in ABA levels in plant tissues enhances adaptation to salinity by promoting osmotic adjustment and stimulating the synthesis of proteins involved in stress tolerance Farooq et al. [2017].

1.1.12 Ion Homeostasis at the Molecular Level

Under salt stress, plants maintain ion homeostasis by regulating ion fluxes in order to limit the accumulation of toxic Na^+ and to promote the uptake of essential K^+. Maintaining a low Na^+/K^+ ratio is essential for proper cytosolic enzyme activity, membrane potential maintenance, and cell volume regulation. At the molecular level, these processes are mediated by Na^+/H^+ antiporters and proton pump-activated transporters (H^+-ATPase and H^+-PPase), which enable Na^+ transport and sequestration into cellular compartments Farooq et al. [2017].

1.1.13 Synthesis of Osmoprotectants

During osmotic stress, plants accumulate specific organic solutes collectively known as compatible solutes. These compounds do not interfere with enzymatic activities even when present at high concentrations. Key osmoprotectants include Abbasi et al. [2016]:

- **Soluble sugars**: Sucrose, glucose, and fructose contribute to osmotic adjustment by maintaining cellular water balance under salt stress Abbasi et al. [2016].
- **Free amino acids**: Several amino acids, including arginine, glycine, alanine, serine, valine, leucine, and proline, accumulate under salt stress and contribute to cellular osmotic adjustment Farooq et al. [2017].
- **Proline**: Proline is the most extensively studied osmolyte in plant responses to salinity. Its accumulation increases markedly under salt stress, playing a crucial role in osmotic adjustment, protection of membrane integrity, stabilization of cellular structures, and reduction of oxidative damage Farooq et al. [2017].

1.1.14 Cell Wall Regulation

The cell wall plays an essential role in plant responses to salt stress by acting as a primary protective barrier and a key element in stress perception. Structurally, the cell wall is mainly composed of cellulose, pectins, and lignin, which directly contribute to mechanisms of salt stress tolerance. The enzyme CCoAOMT1, involved in lignin biosynthesis, is essential for this response, and its loss of function leads to increased sensitivity to salt stress Zhao et al. [2021].

1.1.15 Management Strategies

Improving plant tolerance to salt stress requires an integrated approach combining genetic improvement and agronomic practices. Conventional breeding, molecular tools, and biotechnological approaches enable the development of more tolerant genotypes capable of maintaining ion homeostasis, osmotic adjustment, and antioxidant defence mechanisms. In addition, the use

of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) enhances nutrient uptake, nodulation, photosynthesis, and plant growth under saline conditions. The exogenous application of phytohormones and osmoprotectants, together with seed priming techniques and the appropriate use of biofertilizers and biostimulants, also contributes to improving salt tolerance Farooq et al. [2017].

1.2 Plant Biostimulants

Introduction

Modern agriculture faces the dual challenge of increasing crop productivity to meet the demands of a growing global population while reducing the environmental footprint of intensive farming practices Calvo et al. [2014b]. The overuse of synthetic fertilizers and pesticides has led to soil degradation, nutrient imbalances, reduced microbial diversity, and water contamination Ferreira et al. [2023]. In this context, plant biostimulants have emerged as a promising, eco-friendly alternative to enhance crop resilience and productivity, particularly under abiotic stress conditions such as drought and salinity du Jardin [2015].

According to the 2018 US Farm Bill, a plant biostimulant is defined as a substance or microorganism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stresses, or crop quality and yield. The European Biostimulants Industry Council (EBIC) similarly defines them as products that stimulate natural processes to improve nutrient use efficiency, stress tolerance, and crop quality Xu and Geelen [2018].

This section provides a comprehensive overview of the main categories of biostimulants, with a particular focus on vermicompost, effective microorganisms (EM), and fermented organic formulations. Their mechanisms of action — including modulation of antioxidant defence systems, osmolyte accumulation, and phytohormonal regulation — are discussed in detail, laying the foundation for the experimental investigation of a novel fermented vermicompost-based biostimulant for salt stress mitigation in tomato (*Solanum lycopersicum* L.).

1.3 Categories of Plant Biostimulants

Plant biostimulants can be classified into several categories based on their origin and composition. du Jardin [2015] proposed seven main classes: (1) humic substances (humic and fulvic acids), (2) protein hydrolysates and amino acids, (3) seaweed extracts, (4) chitosan and other biopolymers, (5) inorganic compounds (e.g., silicon), (6) beneficial fungi (e.g., *Trichoderma* spp.), and (7) beneficial bacteria (e.g., *Bacillus*, *Pseudomonas*, *Rhizobium*). More recently, microbial consortia such as Effective Microorganisms (EM) and fermented plant extracts have been added as emerging categories Yakhin et al. [2016].

1.3.1 Humic and Fulvic Acids

Humic substances are complex mixtures of organic compounds formed during the microbial degradation of plant and animal residues. Fulvic acid has a lower molecular weight and higher cation exchange capacity than humic acid, making it more readily absorbed by plants. These substances induce auxin-like effects, stimulate root development, and improve nutrient uptake Canellas et al. [2015]. Trevisan et al. [2011] demonstrated that humic substances affect gene expression related to primary metabolism and development in *Arabidopsis*.

1.3.2 Seaweed Extracts

Seaweed extracts, particularly from *Ascophyllum nodosum* and *Ecklonia maxima*, contain a rich array of bioactive compounds including cytokinins, auxins, gibberellins, betaines, alginates, and polyphenols Sharma et al. [2014]. These compounds enhance seed germination, root development, chlorophyll content, and photosynthetic efficiency. Seaweed extracts also mitigate the effects of abiotic stresses such as salinity, drought, and extreme temperatures Stirk et al. [2014]. Elansary et al. [2020] demonstrated that seaweed extracts significantly enhanced growth and salt tolerance in tomato.

1.3.3 Protein Hydrolysates and Amino Acids

Protein hydrolysates (PHs) are mixtures of polypeptides, oligopeptides, and free amino acids obtained by chemical or enzymatic hydrolysis of animal or plant by-products Xu and Geelen [2018]. Specific amino acids such as glycine betaine and L-proline have well-documented osmoprotectant and antioxidant functions Ashraf and Foolad [2007a]. These compounds stabilize protein structures, scavenge reactive oxygen species (ROS), and maintain membrane integrity under stress conditions Szabados and Savouré [2010].

1.3.4 Silicon

Silicon (Si) is not considered an essential element for most plants, but its beneficial effects under biotic and abiotic stress are well-established Epstein [1999]. Potassium silicate (K_2SiO_3) hydrolyzes in solution to monosilicic acid (H_4SiO_4), which is taken up by plant roots via aquaporins and deposited as amorphous silica in cell walls. This deposition creates physical barriers against pathogens, reduces transpiration, and improves structural integrity Liang et al. [2007].

1.3.5 Melatonin and Thiamine

Melatonin (*N*-acetyl-5-methoxytryptamine) has recently emerged as a potent biostimulant with both antioxidant and growth-regulating properties Arnao and Hernández-Ruiz [2015]. Wang et al. [2012] showed that melatonin triggers defence responses against high light and nitrogen starvation in tomato. Thiamine (vitamin B_1) functions as an activator of systemic acquired resistance (SAR) in plants, protecting against fungal, bacterial, and viral infections.

1.3.6 Chitin and Chitosan

Chitin, a biopolymer from crustacean shells, and its deacetylated form chitosan, have potential applications as biostimulants and biofungicides Pichyangkura and Chadchawan [2015]. Chitosan induces defence responses through the accumulation of H_2O_2 and Ca^{2+} signalling, leading to the activation of phenylalanine ammonia-lyase (PAL) and the accumulation of phenolic compounds Sharif et al. [2018].

1.3.7 Vermicompost: A Multi-Functional Biostimulant

Vermicompost is the product of organic waste decomposition through the joint action of earthworms and microorganisms. It is rich in humic acids, macro- and micronutrients, plant growth regulators (auxins, cytokinins, gibberellins), enzymes, and beneficial microbes Toor et al. [2023].

1.4 Vermicompost as a Biostimulant and Its Mechanisms of Stress Alleviation

Vermicompost enhances plant tolerance to abiotic stress through multiple mechanism (Figure.3) Rehman et al. [2023]:

1. **Improved soil physical properties:** Vermicompost increases soil porosity, aeration, water-holding capacity, and aggregate stability Gutiérrez-Miceli et al. [2007].
2. **Increased nutrient availability:** It supplies nitrates, exchangeable phosphate, soluble potassium, calcium, magnesium, as well as micronutrients such as iron (Fe), zinc (Zn), and copper (Cu) Rehman et al. [2023].
3. **Stimulation of antioxidant defenses:** Abiotic stresses such as salinity lead to overproduction of reactive oxygen species (ROS), including the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^\cdot$). These ROS damage lipids, proteins, and DNA Hasanuzzaman et al. [2020]. Biostimulants enhance the activity of both enzymatic

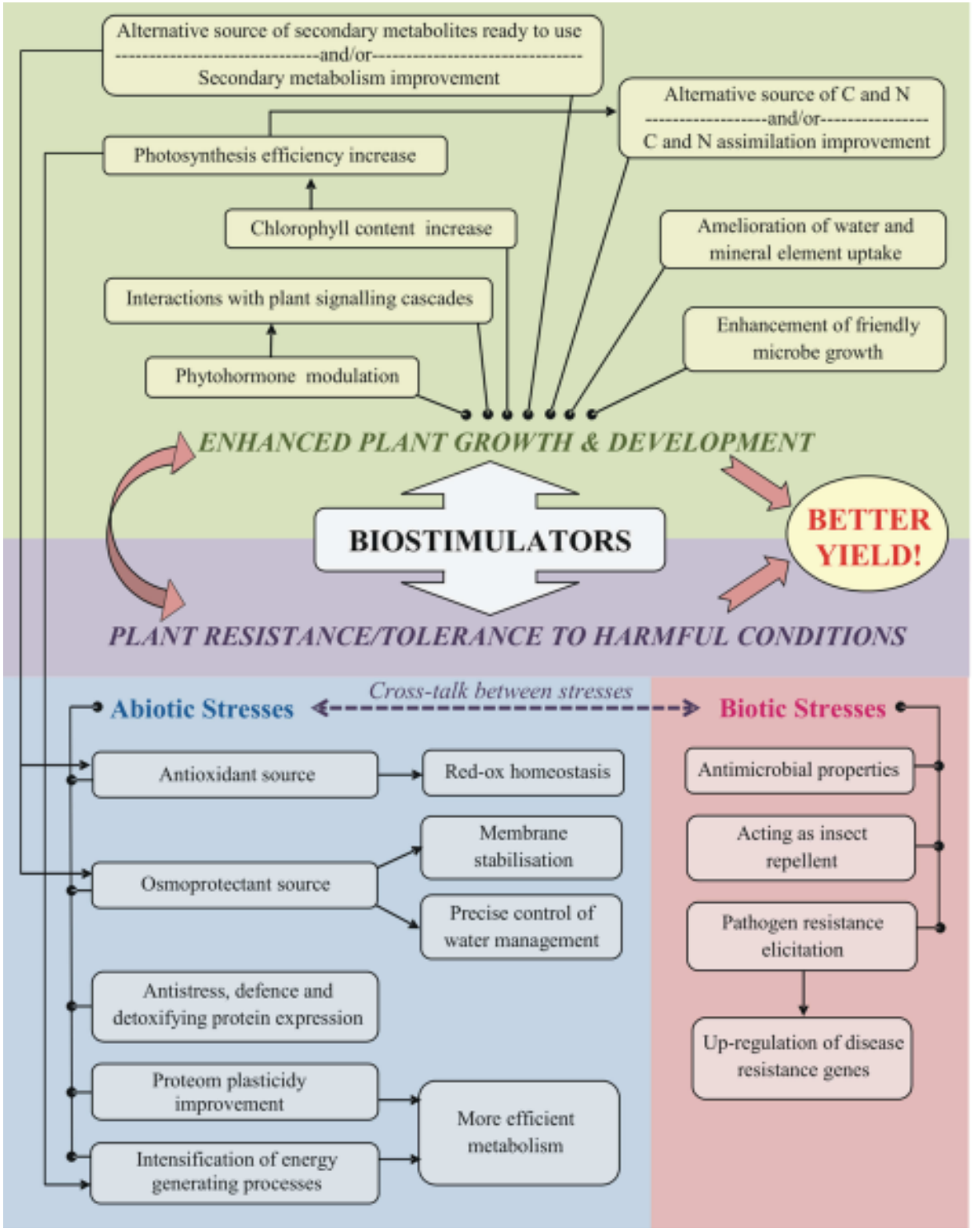


Figure 1.3: Biostimulator modes of action and their correlations

and non-enzymatic antioxidant systems. Ahmad et al. [2022] demonstrated that vermicompost application increases the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and glutathione reductase (GR) in plants subjected to stress, thereby reducing the accumulation of reactive oxygen species (ROS) and lipid peroxidation. Talaat and Abdel-Salam [2024] demonstrated that the combined treatment of vermicompost and Effective Microorganisms (EM) increased the activities of SOD by 285%, CAT by 304%, POD by 224%, APX by 374%, MDHAR by 229%, DHAR by 210%, GR by 371%, and GPX by 155% in wheat subjected to stress. Non-enzymatic antioxidants include ascorbate (AsA), reduced glutathione (GSH), phenolic compounds, flavonoids, carotenoids, and tocopherols. Biostimulants increase the pools of these molecules and maintain high AsA/DHA and GSH/GSSG ratios, which are critical for cellular redox homeostasis Hasanuzzaman et al. [2020].

4. **Osmolyte accumulation:** Osmolytes are small, soluble molecules that accumulate in the cytoplasm under stress conditions, reducing osmotic potential and maintaining turgor without toxic effects Serraj and Sinclair [2006]. Plants treated with vermicompost accumulate higher levels of proline, glycine betaine, soluble sugars, and free amino acids, contributing to the maintenance of cell turgor and membrane stability Hafez et al. [2021]. Talaat and Abdel-Salam [2024] observed increases of 93% in proline, 107% in glycine betaine, and 81% in soluble sugars in biostimulant-treated wheat under stress conditions.

5. **Phytohormonal activity:** Biostimulants contain or stimulate the production of phytohormones. Vermicompost leachates contain cytokinins, auxins, gibberellins, and abscisic acid, which directly modulate plant growth and stress responses Aremu et al. [2015].

6. **Glyoxalase System Activation:** Methylglyoxal (MG) is a cytotoxic byproduct of glycolysis that accumulates under abiotic stress. The glyoxalase system, comprising Glyoxalase I (Gly I) and Glyoxalase II (Gly II), detoxifies MG using GSH as a cofactor Hasanuzzaman et al. [2017]. Biostimulants enhance Gly I and Gly II activities, reducing MG accumulation and its damaging effects. Talaat and Abdel-Salam [2024] showed that vermicompost + EM increased Gly I by 71% and Gly II by 141%, reducing MG by 65% under severe drought.

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1.5 Fermented Organic Biostimulants: A Circular Economy Approach

The valorization of agro-industrial and household waste into biostimulants is fully aligned with the principles of the circular economy Xu and Geelen [2018]. Vilas et al. [2025] developed a fermented plant tonic based on sugarcane bagasse, fruit peels, vegetable waste, coconut water, buttermilk, rice water, and jaggery. After 7–10 days of fermentation at 25–35□, the tonic diluted to 10The fermentation process offers several advantages Di Cagno et al. [2018]:

1. It breaks down complex organic compounds into simpler forms that are readily available to plants.
2. It produces bioactive metabolites, including organic acids, enzymes, and phytohormones.
3. It reduces the volume and pathogen load of organic waste.
4. It enriches the final product with beneficial microorganisms such as lactic acid bacteria, yeasts, and actinomycetes.

1.5.1 Mechanisms of Action of Fermentation-Associated Microorganisms

According to Abd El-Mageed et al. [2022], microorganisms naturally present in vermicompost and further promoted by the fermentation process play an important role in enhancing plant growth and stress tolerance through the following mechanisms:

- **Enhanced nutrient availability:** They solubilize phosphorus, fix atmospheric nitrogen, and decompose organic matter.
- **Production of bioactive substances:** They produce vitamins, amino acids, enzymes, and phytohormones (IAA, GA_3, and cytokinins) Talaat [2015].
- **Upregulation of the antioxidant system:** They increase the activities of SOD, CAT, APX, and GR in plants subjected to stress Abd El-Mageed et al. [2022].
- **Pathogen suppression:** Lactic acid bacteria and actinomycetes produce antimicrobial compounds that inhibit soil-borne pathogens.
- **Improved plant water status:** EM-treated plants maintain higher relative water content and stomatal conductance under drought conditions Iriti et al. [2019].

Chapter 2

Materials and Methods

Introduction

Saline stress constitutes a major threat to plant survival, severely compromising crop growth and yield. In this context, the application of biostimulants, including vermicompost-derived formulations, represents a promising alternative for meeting the demands of sustainable agriculture.

2.1 Research Objectives

The primary objective of this research is to evaluate and compare the effects of three fermented organic formulations — a vermicompost tea-based fermentation (VF), a fermentation of aromatic and spontaneous plant species (*Eucalyptus* spp., *Cynodon dactylon*, *Urtica dioica*, *Rosmarinus officinalis*, *Inula* spp., and *Sinapis arvensis*) (PF), and a combined fermentation of vermicompost tea and these plant species (VPF) — on the following parameters of tomato (*Solanum lycopersicum* L.) plants subjected to salt stress:

- Growth parameters: cumulative aerial growth, root growth, aerial biomass, root biomass, and leaf number;
- Photosynthetic pigments: chlorophylls a, b, and total chlorophyll, as well as carotenoids;
- Phenolic and flavonoid compounds;
- Compatible solute accumulation: proline and total soluble sugars.

This study also aims to evaluate and compare microbial activity across the different fermented formulations.

2.2 Biological Study Material

2.2.1 Description of the Growth Chamber

The experiment was conducted in a controlled-environment greenhouse belonging to the Department of Agronomy, Faculty of Sciences, University Pole of Ouzera, Médéa. The greenhouse covers a surface area of 128 m^2, with dimensions of 16 m in length, 30 m in width, and 15 m in height (Figure 2.1). Internal temperature was maintained between 25 °C and 35 °C, while relative humidity ranged from 50% to 70%.



Figure 2.1: The controlled-environment greenhouse at the University Pole of Ouzera, Médéa (Original, 2026).

2.2.2 Tomato Seedling Preparation

Experiments were conducted on seedlings of tomato (*Solanum lycopersicum* L.), variety Super Marmande, selected for its early maturity and vigour. Prior to sowing, seeds were surface-sterilised by immersion for five minutes in a 1% sodium hypochlorite solution to eliminate surface contaminants, followed by several rinses in distilled water to remove residual chlorine. Seeds were then sown in plastic plug trays filled with peat. Once seedlings reached the five-to-six-leaf stage, they were carefully transplanted into plastic pots containing a substrate composed of equal parts sand, soil, and peat (1:1:1), previously autoclaved at 121 °C for 90 minutes. Plants were irrigated regularly throughout the duration of the trial.



Figure 2.2: The successive stages of tomato plant cultivation from seed germination to transplanting (Original, 2026).

2.2.3 Collection and Selection of Plant Species

For this study, plant-derived organic materials were selected on the basis of local availability and their recognised potential as sources of bioactive compounds with biostimulant properties. Priority was given to aromatic, medicinal, and spontaneous plant species widely distributed in the Médéa region.

The following species were used:

- *Eucalyptus* spp. (eucalyptus or gum tree);
- *Cynodon dactylon* (Bermuda grass or couch grass);
- *Urtica dioica* (common stinging nettle);
- *Rosmarinus officinalis* (rosemary);
- *Inula* spp. (fleabanes);
- *Sinapis arvensis* (wild mustard).

These plants were harvested from the Médéa region. Their selection was based on local availability, environmental adaptability, and their documented richness in secondary metabolites likely to contribute to the biostimulant activity of the fermented extracts.

2.2.4 Vermicompost Production

Vermicomposting was carried out at the laboratory of the University Dr. Yahia Farès de Médéa over a period of five months. The vermicompost was produced through the bioconversion of solid and liquid organic matter by the epigeic earthworm species *Eisenia fetida* (Savigny, 1826), procured from a breeder in the Bou Saâda region, Wilaya of M'Sila.

Vertical wooden vermicomposting units were used for production. These devices were placed in a dark, well-ventilated environment under conditions optimised for earthworm activity, namely a temperature of 15–20 °C, relative humidity of 65–70%, and a pH of 6–7.

The vermicomposting process yields two distinct products: vermicompost leachate (or worm juice), a liquid resulting from the degradation of organic matter by earthworms and associated microorganisms, and vermicompost tea, a liquid solution rich in mineral elements and organic compounds obtained by extraction from the solid vermicompost fraction. The present study focused primarily on the solid vermicompost fraction.

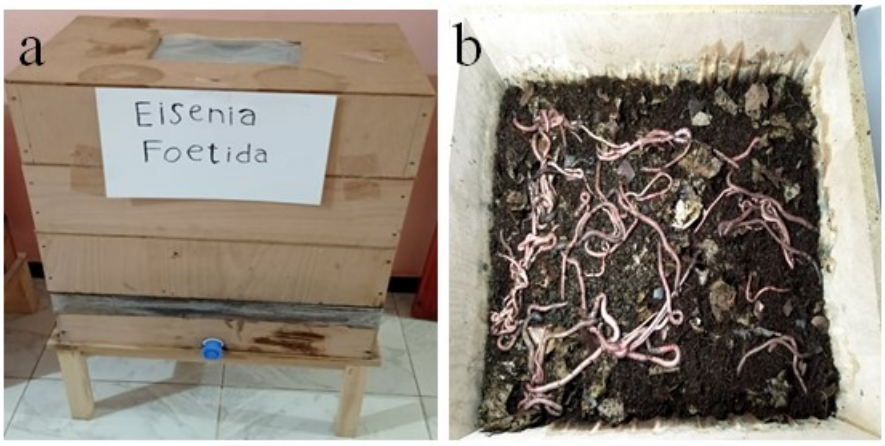


Figure 2.3: Vermicompost production process (Original, 2026).

2.3 Experimental Methods

2.3.1 Preparation of Fermented Formulations

Fermented formulations were prepared by macerating 1 kg of organic material in 10 L of water. Three formulations were produced as follows:

- **Formulation 1 (F1):** 1 kg of vermicompost tea in 10 L of water.
- **Formulation 2 (F2):** 1 kg of plant material (*Eucalyptus* spp., *Cynodon dactylon*, *Urtica dioica*, *Rosmarinus officinalis*, *Inula* spp., and *Sinapis arvensis*, at variable proportions) in 10 L of water.
- **Formulation 3 (F3):** 500 g of vermicompost and 500 g of the plant mixture (as above) in 10 L of water.

Formulations were fermented in plastic containers for one month at ambient temperature, with regular stirring every two to three days to ensure aeration and homogenisation. Upon completion of the incubation period, solutions were filtered through clean cloth to recover the enriched liquid fraction.

Prior to application, each formulation was diluted to 5% (D5) and 10% (D10). Both concentrations were applied to tomato plants subjected to salt stress in order to evaluate their efficacy on growth parameters and phytochemical production.



Figure 2.4: The three formulations (Original, 2026).

2.3.2 Experimental Design and Treatment Application

The trial was conducted under greenhouse conditions using a completely randomised block design. The design comprised seven blocks, each containing 30 plants, for a total of 210 plants. Each experimental block received one of the following root-applied biostimulant treatments:

- **Block 1:** fermented vermicompost diluted to 5%;
- **Block 2:** fermented vermicompost diluted to 10%;
- **Block 3:** fermented plant extract diluted to 5%;
- **Block 4:** fermented plant extract diluted to 10%;
- **Block 5:** combined vermicompost and plant fermentation diluted to 5%;
- **Block 6:** combined vermicompost and plant fermentation diluted to 10%;
- **Block 7:** control (tap water).

Biostimulants were applied via root irrigation using a 250 mL container to ensure a uniform volume across all plants. Plants were watered regularly according to their hydric requirements.

Biostimulant treatments were applied once per week during the first three weeks of the experiment. Thereafter, tomato plants subjected to saline stress were irrigated twice weekly with 100 mL of NaCl solutions at progressively increasing concentrations (150, 200, 250, and 300 mM).

Four to five plants per block were randomly sampled each week, both before and after stress application, to assess the effect of biostimulants on plant tolerance to saline stress. The experimental monitoring period spanned six weeks.

From the collected plants, the following morphological parameters were assessed: aerial and

root length growth, fresh biomass of aerial and root parts, and leaf number. Sampled plants were also used for the extraction and quantification of phytochemical compounds, including flavonoids, polyphenols, chlorophyll, proline, and total sugars.

2.3.3 Assessment of Microbial Activity in the Fermented Formulations

2.3.3.1 Preparation of Culture Medium

To assess microbial activity, considered the primary driver of the fermentation process, a Potato Dextrose Agar (PDA) culture medium was prepared in the laboratory at a pH of 6.5–7, following the protocol described by Impion [2010].

The preparation procedure was as follows:

- **Weighing and dissolution:** The required quantity of PDA powder was precisely weighed on an analytical balance and dissolved in a predefined volume of distilled water, in accordance with the manufacturer's recommendations.
- **Sterilisation:** The prepared medium was autoclaved at 121 °C for 20 minutes to ensure aseptic conditions.
- **Plating:** After cooling to approximately 45 °C, the medium was poured under a laminar flow hood into sterile Petri dishes at approximately 10 mL per dish to achieve a depth of approximately 0.5 cm. Dishes were left to solidify completely before use.



Figure 2.5: preparation of Culture Medium PDA (Original, 2026).

2.3.3.2 Estimation of Microbial Population Density

Microbial population density was estimated using serial dilutions and plating on PDA medium. A volume of 90 mL of sterile distilled water (autoclaved at 121 °C for 15–20 minutes) was prepared, to which 10 mL of fermented material — sampled from collection traps every two days — was added to obtain a one-tenth dilution (10^{-1}). The suspension was homogenised by agitation.

From this initial suspension, serial dilutions were prepared to obtain concentrations of 10^{-2}, 10^{-3}, and 10^{-4} by successive 1-in-10 dilutions.

Aliquots from each dilution were then spread onto sterile PDA-filled Petri dishes. Cultures were incubated at 28 °C for 24 hours for bacteria and 48–72 hours for fungi. Following incubation, microbial colonies were enumerated and population density was calculated for each formulation.

This procedure was repeated across all formulations to compare microbial activity among the different fermented substrates.



Figure 2.6: preparation of Serial Dilution and plating on PDA (Original, 2026).

2.4 Measured Parameters

2.4.1 Aerial and Root Length Growth

Aerial and root length were measured after each sampling event using a ruler. Both the aerial part (from the soil surface to the apical meristem) and the root system were measured separately.

2.4.2 Aerial and Root Fresh Biomass

The fresh biomass of the aerial and root fractions was determined in milligrams by separately weighing each part on a precision analytical balance.

2.4.3 Leaf Number

The total number of leaves per plant was counted for each treatment at each sampling time.

2.4.4 Biochemical Parameter Assessment

2.4.4.1 Proline Quantification

Proline content was determined following the colorimetric method of Troll and Lindsley [1955]. Briefly, 100 mg of fresh plant material was ground and homogenised in 2 mL of methanol, then heated at 85 °C for 60 minutes. After cooling, acetic acid, ninhydrin reagent, and a mixture of distilled water, acetic acid, and orthophosphoric acid (0.4:1:0.26) were added to the extract, and the solution was brought to the boil for 30 minutes, yielding a red coloration. After cooling, toluene was added to induce phase separation; the upper toluene phase containing proline was collected, dried over anhydrous Na_2SO_4, and absorbance was read at 528 nm. Proline concentration was determined from a standard curve according to the following linear equation:

$$Y = 0.1043\,X \tag{2.1}$$

where Y is absorbance (nm) and X is L-proline concentration (mg mL^{-1}).

2.4.4.2 Total Soluble Sugar Quantification

Total soluble sugars were quantified using the phenol–sulfuric acid method of Dubois et al. [1956]. Plant material was contacted with 80% ethanol for 48 hours at ambient temperature. The alcoholic extract was evaporated in an oven at 80 °C, and the residue was resuspended in 20 mL of distilled water. A 2 mL aliquot of the resulting solution was mixed with 5% phenol and concentrated sulfuric acid (96%), vortexed, then incubated for 10 minutes in a water bath at 30 °C for 20 minutes. Absorbance was read at 485 nm after 10 minutes.

Sugar concentration was determined from a standard curve using the following equation:

$$Y = 4.3918\,X - 0.1946 \tag{2.2}$$

where Y is absorbance (nm) and X is total sugar concentration (mg mL^{-1}).

2.4.4.3 Total Chlorophyll and Carotenoid Quantification

Photosynthetic pigments were quantified following the spectrophotometric method of Lichtenthaler [1987]. Fresh leaf tissue (0.1 g) was homogenised in 4 mL of 80% acetone and centrifuged at 3,000 rpm for 10 minutes. The supernatant was collected and absorbance was measured at 647, 664, and 470 nm using a UV–Vis spectrophotometer.

Chlorophyll and carotenoid contents (expressed in mg g^{-1} DM) were calculated using the fol-

lowing equations:

$$\text{Chl}\, a = 12.21\,(A_{664}) - 2.79\,(A_{647}) \tag{2.3}$$

$$\text{Chl}\, b = 21.21\,(A_{647}) - 5.1\,(A_{664}) \tag{2.4}$$

$$\text{Chl}\, T = \text{Chl}\, a + \text{Chl}\, b \tag{2.5}$$

$$\text{Carotenoids} = \frac{1000 \times A_{470} - 1.8 \times \text{Chl}\, a - 85.02 \times \text{Chl}\, b}{198} \tag{2.6}$$



Figure 2.7: Overview of the chlorophyll extraction and estimation protocol (Original, 2026).

2.4.4.4 Polyphenol Extraction and Quantification

Total polyphenol content was assessed using the Folin–Ciocalteu method described by Singleton and Rossi [1965]. Aqueous plant extracts (200 µL) from treated and control plants were pipetted into test tubes, to which 1 mL of Folin–Ciocalteu reagent (diluted 1:10) and 800 µL of 7.5% sodium carbonate solution were added. Tubes were mixed and incubated in the dark for 30

minutes. Absorbance was then read at 760 nm using a UV–Vis spectrophotometer. A gallic acid standard curve was prepared at concentrations ranging from 0 to 1,000 µg mL^{-1}.



Figure 2.8: Overview of the polyphenol extraction and estimation protocol (Original, 2026).

2.4.4.5 Flavonoid Extraction and Quantification

Flavonoid content was determined by the aluminium chloride colorimetric method, based on the stable complex formed between $AlCl_3$ and the oxygen groups present on the carbon skeleton of flavonoids. The procedure followed protocols adapted from Zhishen et al. [1999] and Kim et al. [2003]. Briefly, 400 µL of plant extract was mixed with 120 µL of 5% $NaNO_2$ solution. After 5 minutes, 120 µL of 10% $AlCl_3$ was added and the mixture was vortexed. After a further 6 minutes, 800 µL of 1 M NaOH was introduced. Absorbance was read at 510 nm. A quercetin standard curve in methanol was prepared at concentrations ranging from 0 to 1,000 µg mL^{-1}.

2.5 Statistical Analysis

Statistical analyses were conducted to evaluate the effects of the biostimulant formulations on the vegetative growth and metabolic activities of tomato plants subjected to salt stress. Analyses

of variance were performed on homogeneous data series, retained on the basis of a coefficient of variation below 15% (CV $<$ 15%).

Growth and phytochemical parameters were presented as histograms generated using PAST software. A General Linear Model (GLM) analysis of variance was applied to identify significant differences between treatments at a threshold of $p \leq 0.05$. All statistical analyses were performed using SYSTAT, version 12.

Chapter 3

Results

3.1 Presentation and Analysis of Obtained Results

3.1.1 Evaluation of the Stimulatory Effect of Bio-stimulants on the Growth Parameters of Tomato under Saline Stress

3.1.1.1 Evolution of the Effect of Bio-stimulants on the Cumulative Aerial Growth of Tomato Seedlings under Saline Stress

The results presented in Figure 3.1 demonstrate that the bio-stimulant factor exerts a significant effect on the aerial growth of tomato seedlings subjected to saline stress, in comparison with the untreated control (GLM test: $p < 0.05$). Post-hoc analysis enabled the identification of two homogeneous groups corresponding to distinct levels of stimulatory activity. The first group, associated with homogeneous group **(a)**, comprises the bio-stimulant treatments that induced the highest growth values. The second group, associated with homogeneous group **(b)**, corresponds to the control, which recorded the lowest growth values among all treatments tested.

The results further indicate that the bio-stimulants based on vermicompost combined with fermented plants at dose D5, as well as fermented vermicompost alone at dose D10, induced the highest values of cumulative aerial growth. These were followed by the plant-based bio-stimulants, whereas the control recorded the lowest growth values among all treatments evaluated.

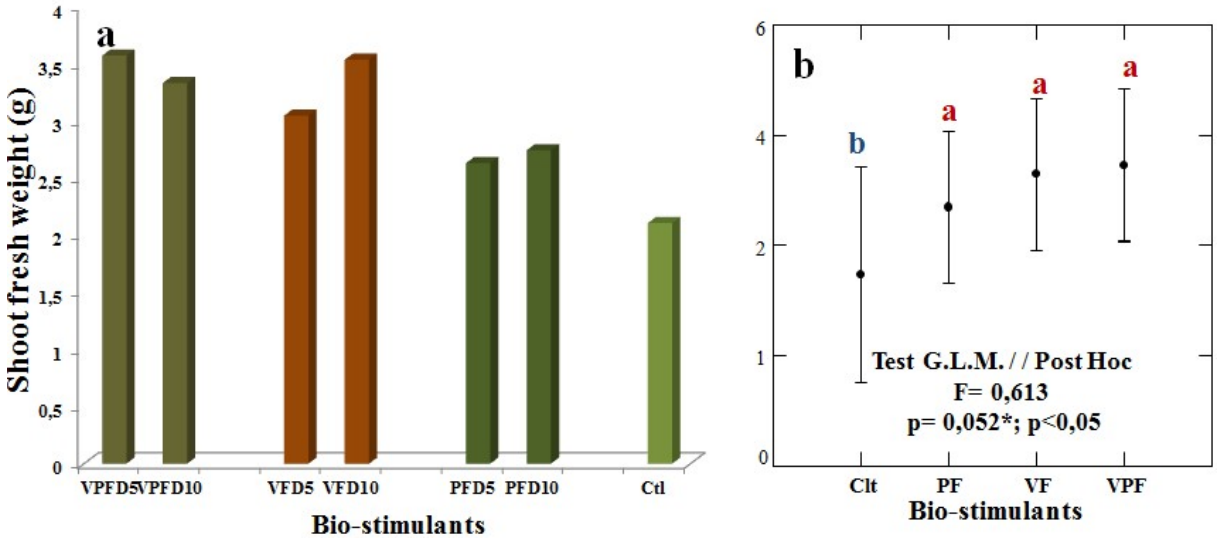


Figure 3.1: Comparative effect of different dilutions of bio-stimulants on the aerial growth of tomato plants subjected to saline stress. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

3.1.1.2 Evolution of the Effect of Bio-stimulants on the Root Growth of Tomato Seedlings under Saline Stress

The graphical representation (Figure 3.2a and b) highlights the effect of different bio-stimulant concentrations on the root growth of tomato seedlings subjected to saline stress.

According to the GLM test results, the different dilutions applied did not exert a statistically significant effect on the root growth of the treated seedlings (GLM test: $p > 0.05$). Despite the absence of a statistically significant difference, the results reveal a tendency towards increased root growth in treated plants compared to the control.

The highest values were recorded in seedlings treated with the bio-stimulant based on vermicompost combined with fermented plants, followed by fermented vermicompost, and then the plant-based bio-stimulants. In contrast, the control recorded the lowest root growth values among all treatments tested (Figure 3.2a and b).

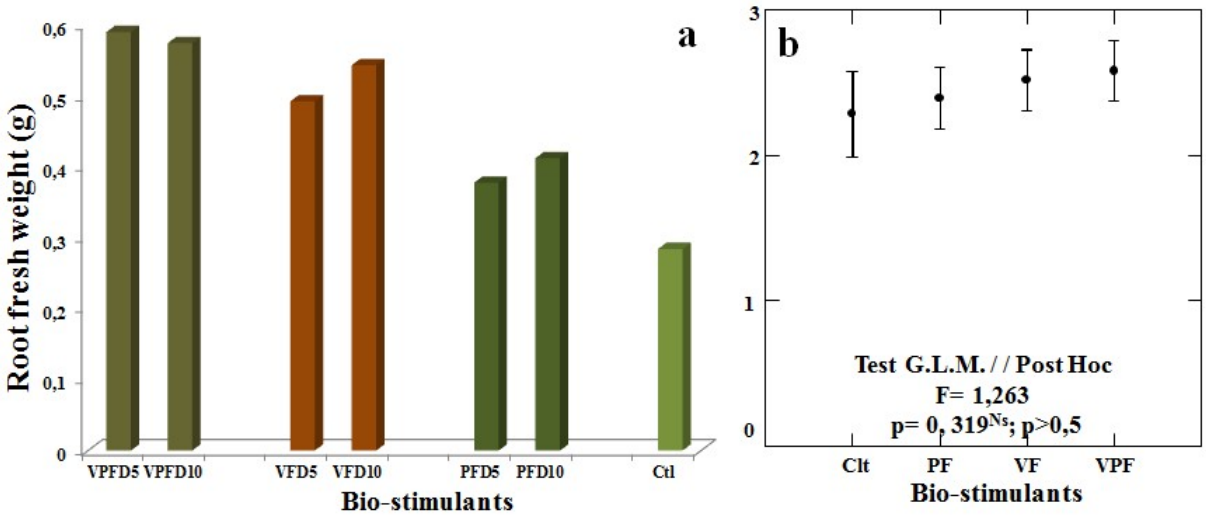


Figure 3.2: Comparative effect of different dilutions of bio-stimulants on the root growth of tomato plants subjected to saline stress. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

3.1.1.3 Evolution of the Stimulatory Effect of Bio-stimulants on the Aerial Biomass of Tomato Seedlings under Saline Stress

The results of the analyses conducted using the GLM model indicate that aerial biomass was not significantly influenced by the different bio-stimulant dilutions applied (GLM test: $p > 0.05$). Nevertheless, the results reveal a tendency towards increased aerial biomass in treated plants relative to the control (Figure 3.3b).

The highest aerial biomass values were observed in seedlings treated with the fermented vermicompost-based bio-stimulant, followed by those treated with plant-based bio-stimulants, and then by those

receiving the combined vermicompost and fermented plants formulation. Furthermore, the control recorded the lowest aerial biomass values among all treatments evaluated (Figure 3.3a and b).

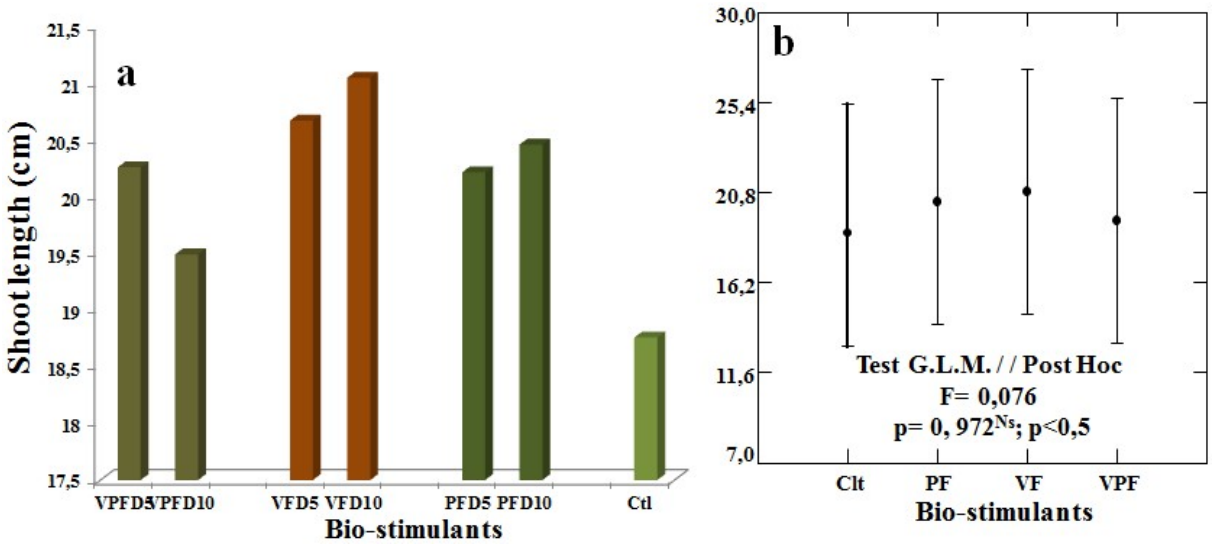


Figure 3.3: Comparative effect of different dilutions of bio-stimulants on the aerial biomass of tomato plants subjected to saline stress. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

3.1.1.4 Evolution of the Stimulatory Effect of Bio-stimulants on the Root Biomass of Tomato Seedlings under Saline Stress

Analyses carried out using the GLM model demonstrate that root biomass is significantly influenced by the different bio-stimulant formulations applied (GLM test: $p < 0.05$). The highest root biomass was recorded in seedlings treated with vermicompost combined with fermented plants (homogeneous group **a**), followed by the fermented vermicompost-based bio-stimulant, and then by the plant-based formulations, corresponding to homogeneous group **b**. The control, whilst exhibiting the lowest root biomass values, constitutes homogeneous group **c** (Figure 3.4a and b).

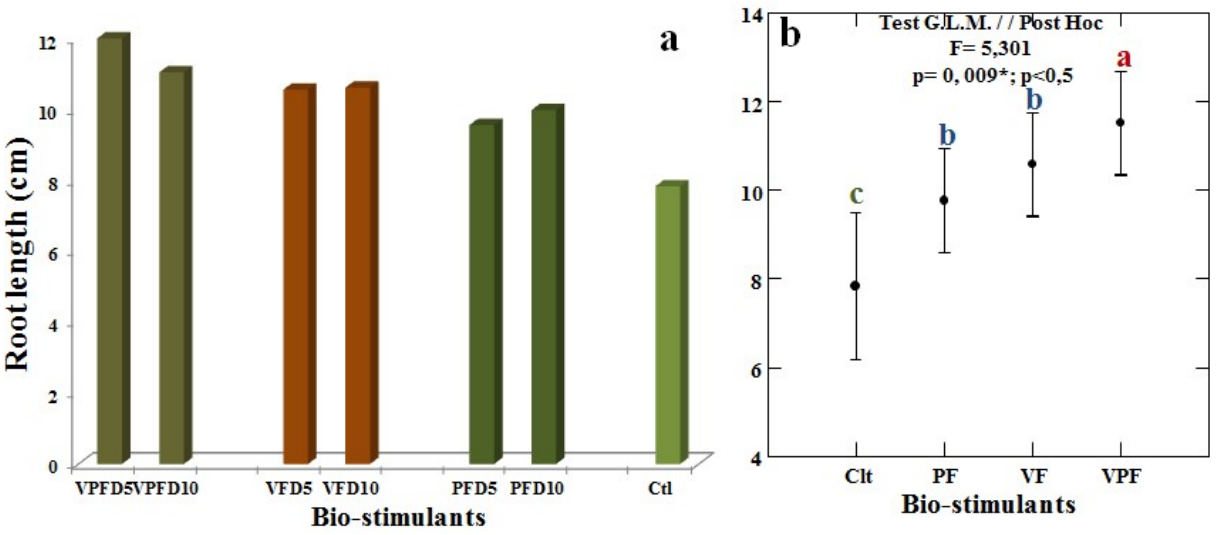


Figure 3.4: Comparative effect of different dilutions of bio-stimulants on the root biomass of tomato plants subjected to saline stress. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

3.1.1.5 Evolution of the Stimulatory Effect of Aqueous Extracts on the Leaf Number of Tomato Seedlings under Saline Stress

The results demonstrate that leaf number was at its maximum following the application of bio-stimulant extracts, whereas the lowest values were observed in the control (Figure 3.5a).

Analyses performed using the GLM model confirm that leaf number (LN) is significantly influenced by the different bio-stimulants tested (GLM test: $p < 0.05$). Post-hoc analysis revealed the existence of two homogeneous groups corresponding to distinct levels of stimulatory activity. The first group, associated with homogeneous group **(a)**, encompasses bio-stimulant-treated plants, while the second group, associated with homogeneous group **(ab)**, comprises untreated plants (Figure 3.5b).

The highest leaf number was recorded in tomato seedlings treated with the bio-stimulant based on vermicompost combined with fermented plants, followed by those treated with fermented vermicompost, and then by those receiving plant-based bio-stimulants. In contrast, the control recorded the lowest leaf number values among all treatments tested (Figure 3.5a and b).

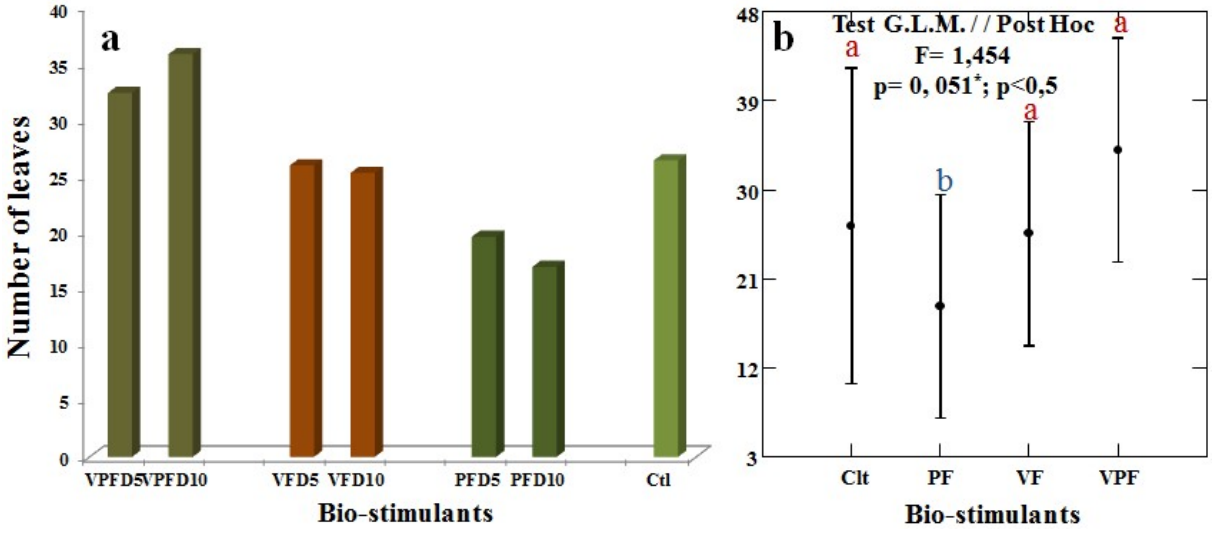


Figure 3.5: Comparative effect of different dilutions of bio-stimulants on the leaf number of tomato plants subjected to saline stress. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

3.1.1.6 Comparative Effects of Bio-stimulants on the Morphological Variation of Tomato Seedlings under Stress Conditions

Figure 3.6 presents a Principal Component Analysis (PCA) of the morphological parameters measured in tomato seedlings subjected to different bio-stimulant treatments under saline stress conditions. The PCA was conducted with the objective of integrating all assessed growth parameters and elucidating the relationships between treatments and morphological variables.

The analysis is considered satisfactory, as more than 90% of the total variance is accounted for by the first two principal axes (Figure 3.6). The PCA biplot reveals a clear separation between the bio-stimulant treatments and the control. Growth variables are primarily associated with seedlings treated with the vermicompost combined with fermented plants bio-stimulant (VPFD5 and VPFD10), as well as with the fermented vermicompost alone (VFD5 and VFD10). In contrast, the plant-based fermentation treatments (PFD5 and PFD10) occupy an intermediate position, reflecting more moderate or variable responses depending on the parameters considered.

The control (Ctl) is clearly distinct from all other treatments, indicating an overall weaker response across all growth parameters studied, namely cumulative aerial growth, root growth, aerial biomass, root biomass, and leaf number.

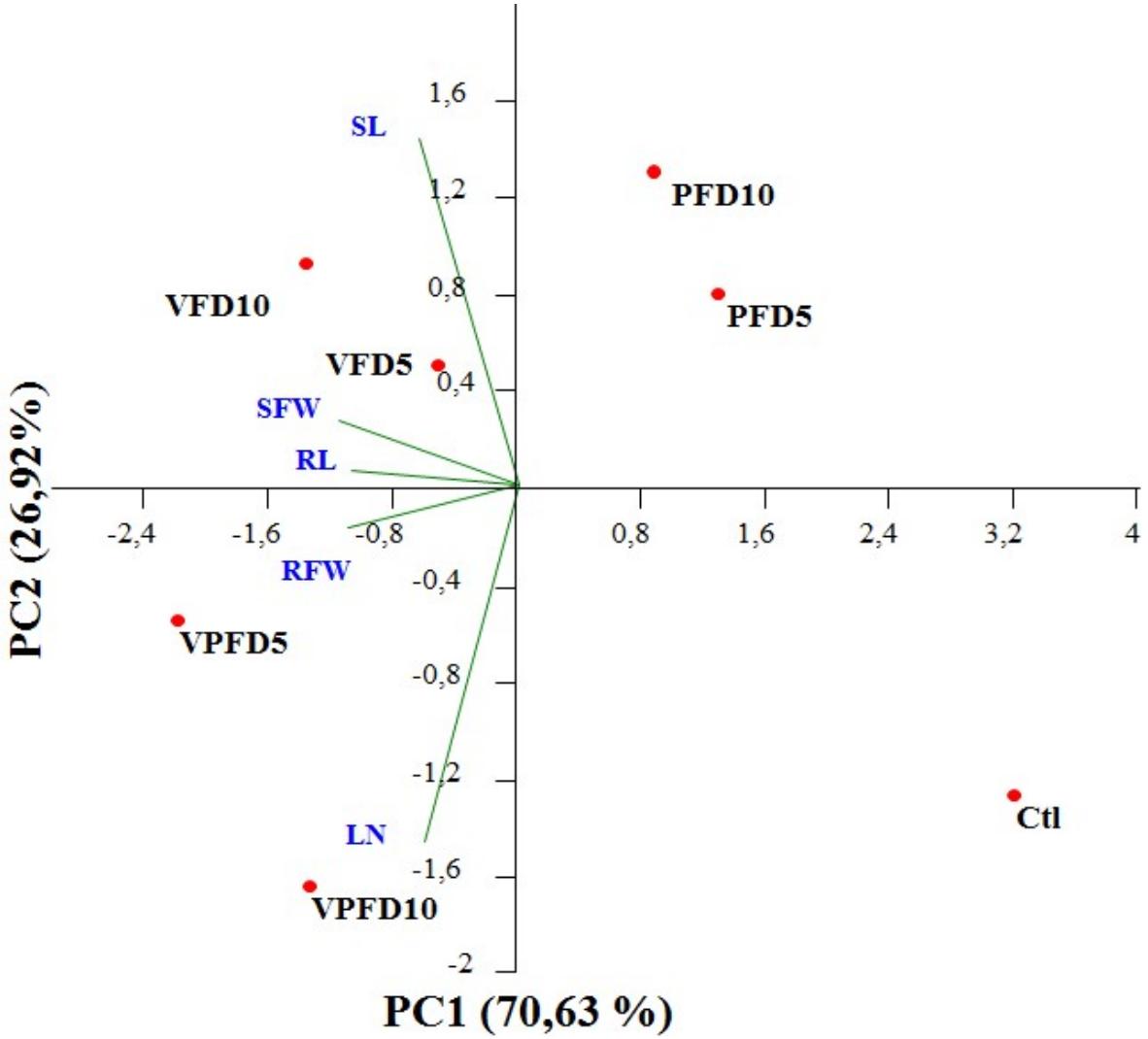


Figure 3.6: Projection of the morphological variation of tomato under the effect of bio-stimulants onto the two principal axes of the PCA. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

3.1.2 Physio-Biochemical Parameters of Tomato Seedlings under Saline Stress

3.1.2.1 Evolution of the Stimulatory Effect of Bio-stimulants on the Chlorophyll Content of Tomato Plants under Saline Stress

Chlorophyll *a* Chlorophyll *a* concentrations measured in tomato seedlings varied according to the bio-stimulant applied and were markedly distinct from those recorded in the untreated control. The results indicate that treatment with vermicompost combined with fermented plants induced the highest chlorophyll *a* contents, followed by fermented vermicompost, whilst the plant-based formulations recorded the lowest values among the treatments tested (Figures 3.7a and b).

Furthermore, the dose effect demonstrated a superior response at the lower concentration D5 (5%), which yielded the highest chlorophyll *a* content compared to dose D10 (10%) (Figure 3.7c).

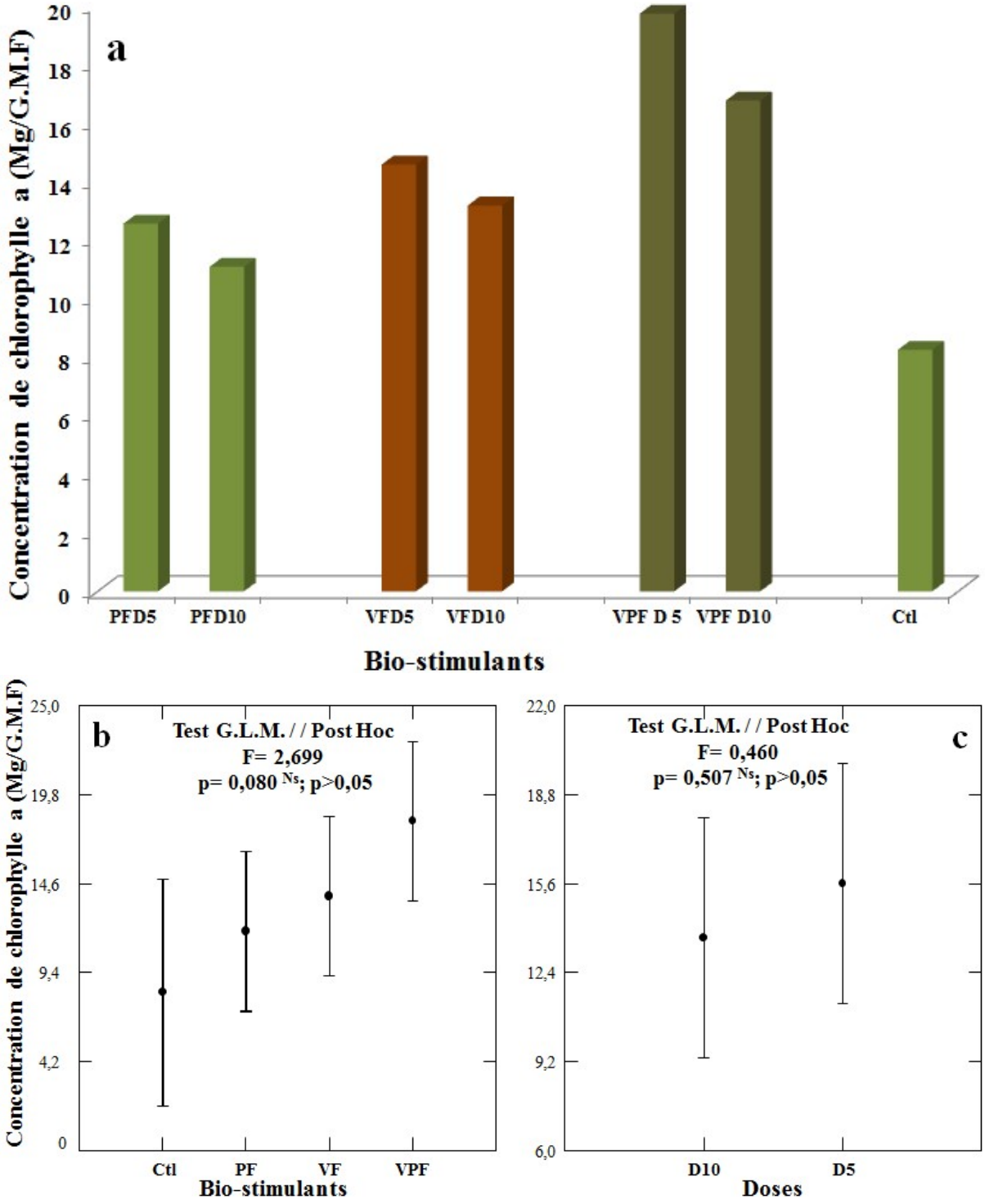


Figure 3.7: Effect of different dilutions of bio-stimulants on chlorophyll *a* concentration in tomato under saline stress. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

Chlorophyll *b* Chlorophyll *b* contents recorded in tomato seedlings showed variation according to the bio-stimulant applied and clearly differed from those observed in the untreated control. The results indicate that treatment with vermicompost combined with fermented plants promoted the highest chlorophyll *b* contents. This treatment was followed by fermented vermicompost and plant-based formulations, which displayed relatively similar effects on the accumulation of this pigment (Figures 3.8a and b).

Moreover, the dose effect revealed superior efficacy at the lower concentration D5 (5%), which yielded the highest chlorophyll *b* content, compared to dose D10 (10%) (Figure 3.8c).

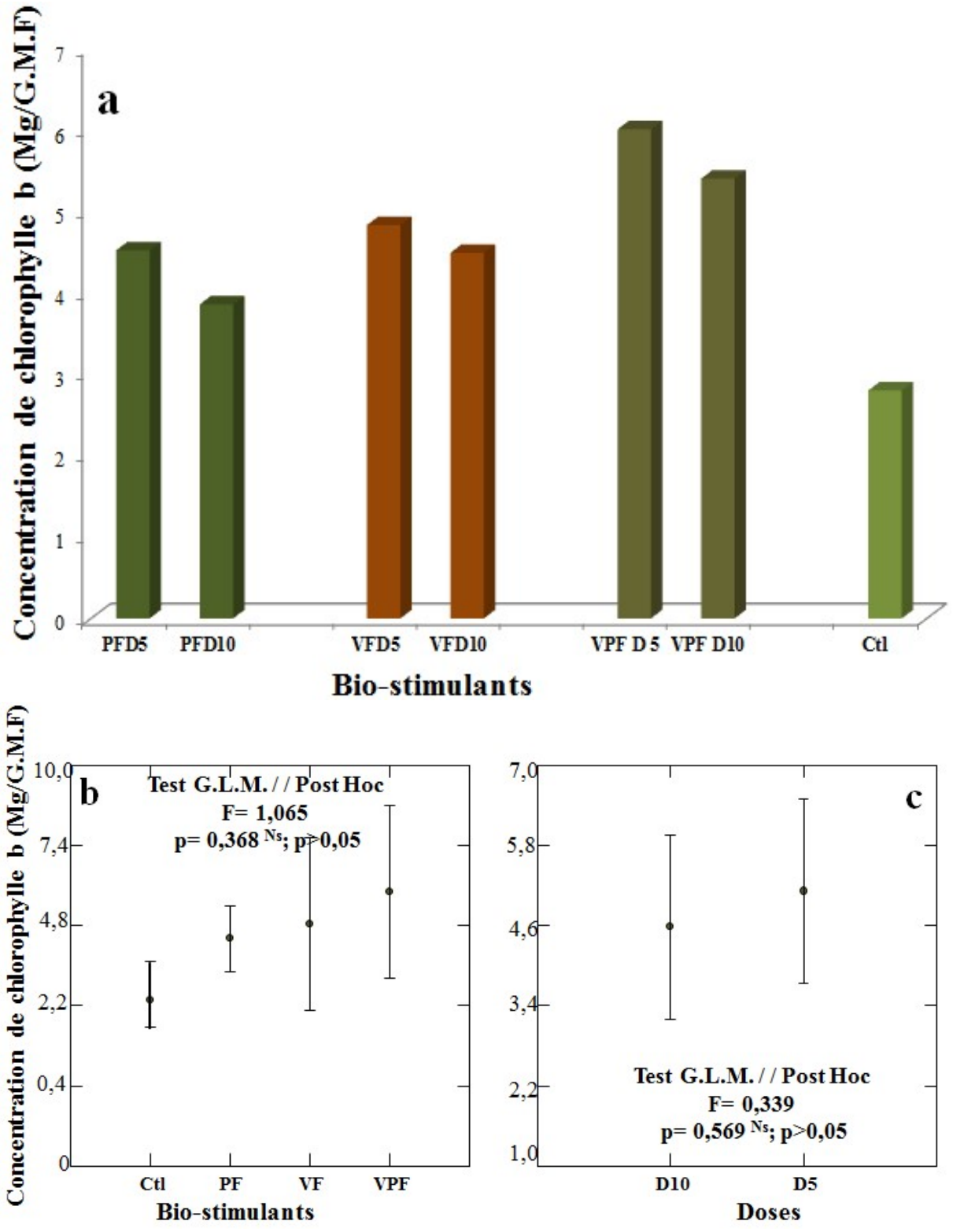


Figure 3.8: Effect of different dilutions of bio-stimulants on chlorophyll *b* concentration in tomato under saline stress. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

Total Chlorophyll Total chlorophyll contents recorded in tomato seedlings varied according to the bio-stimulant applied and were markedly distinct from those observed in the untreated control. The results show that treatment with vermicompost combined with fermented plants induced the highest total chlorophyll contents. This treatment was followed by fermented vermicompost and plant-based formulations, which exhibited relatively similar effects on the accumulation of this pigment (Figures 3.9a and b).

However, the dose effect demonstrated superior efficacy at the lower concentration D5 (5%), which yielded the highest total chlorophyll content compared to dose D10 (10%) (Figure 3.9c).

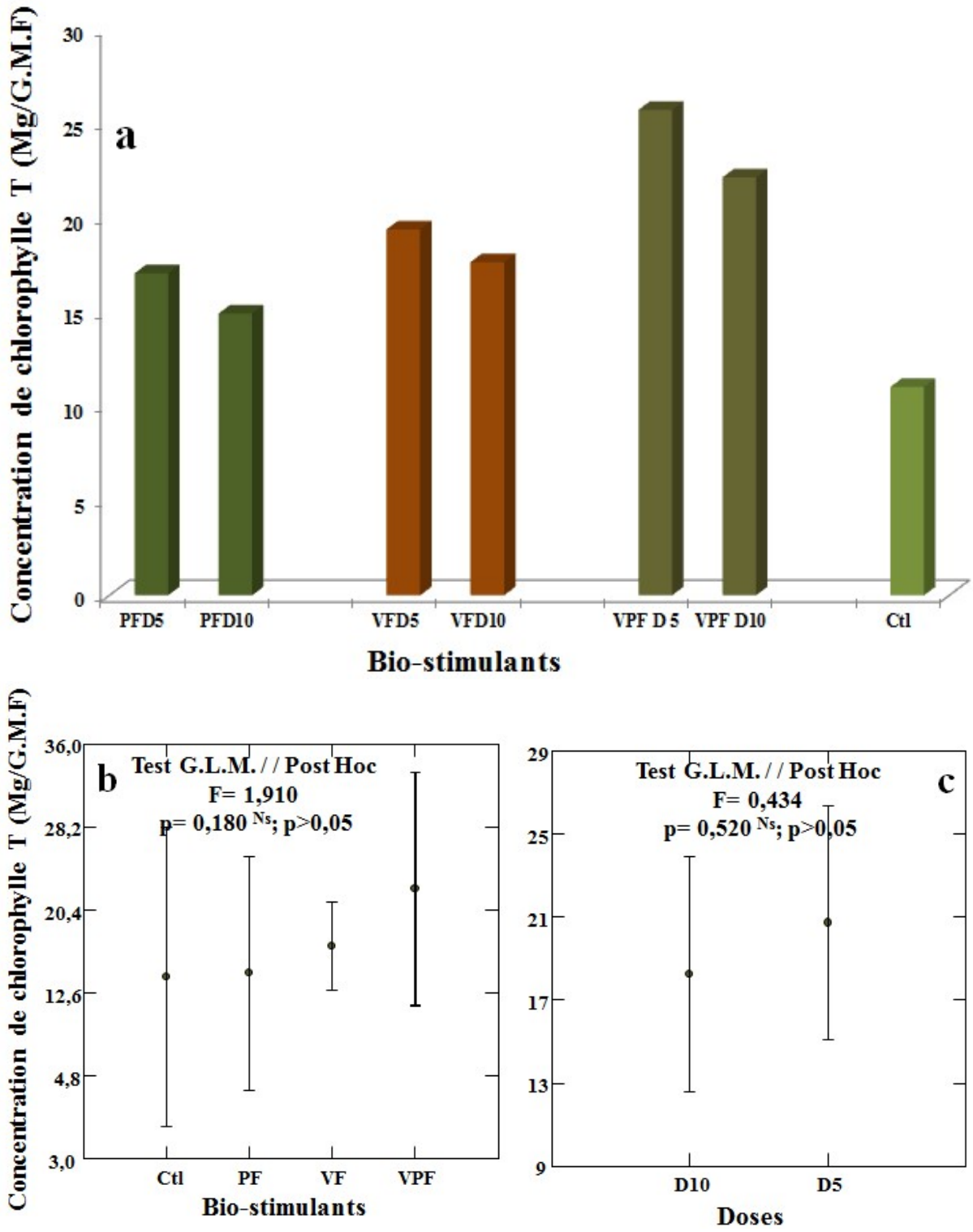


Figure 3.9: Effect of different dilutions of bio-stimulants on total chlorophyll concentration in tomato under saline stress. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

3.1.2.2 Evolution of the Stimulatory Effect of Bio-stimulants on the Total Polyphenol Content of Tomato Plants under Saline Stress

Total polyphenol contents measured in tomato seedlings varied according to the bio-stimulant applied and were markedly distinct from those observed in the untreated control. The results indicate that the highest polyphenol values were recorded in seedlings treated with the fermented vermicompost-based bio-stimulant, followed by those treated with vermicompost combined with fermented plants, and then by the plant-based bio-stimulant formulations. In contrast, the control exhibited the lowest polyphenol contents among all treatments tested (Figure 3.10).

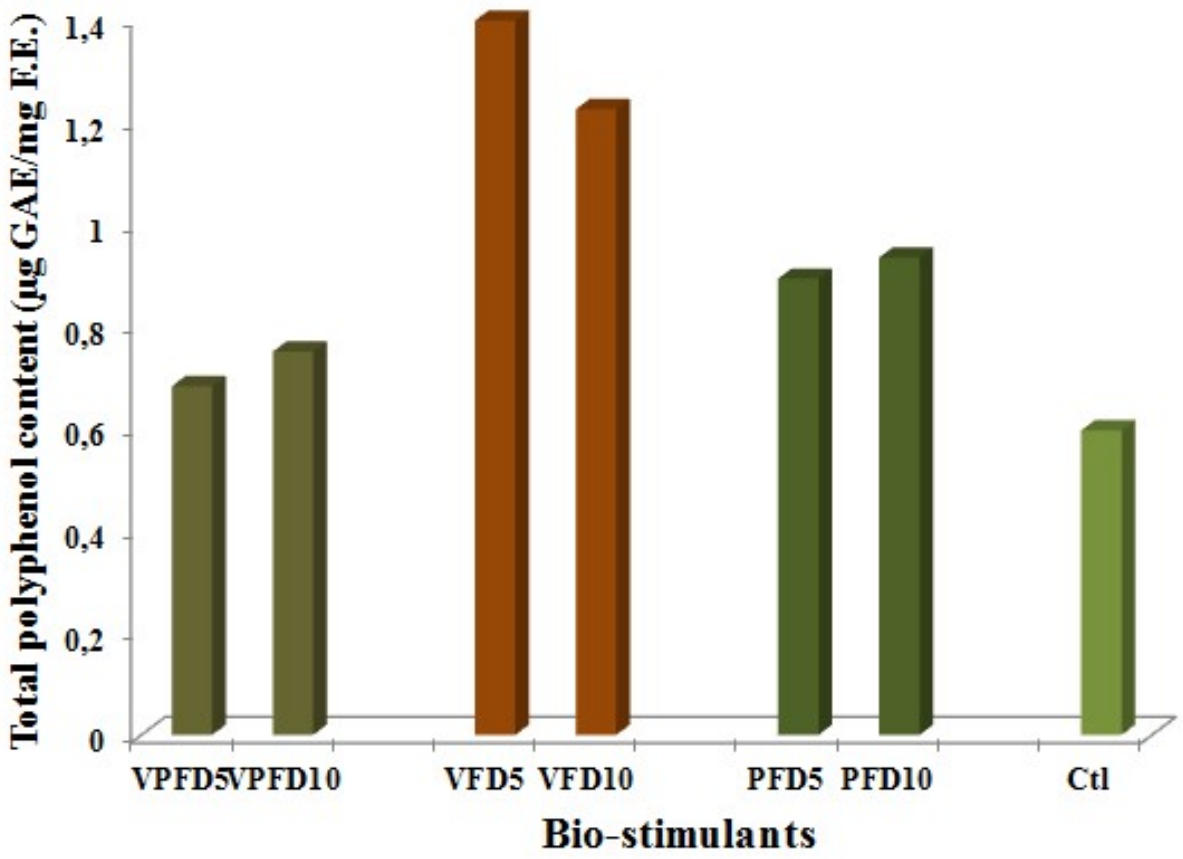


Figure 3.10: Effect of different dilutions of bio-stimulants on total polyphenol content in tomato subjected to saline stress. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

3.1.2.3 Evolution of the Stimulatory Effect of Bio-stimulants on the Flavonoid Content of Tomato Plants under Saline Stress

Flavonoid contents measured in tomato seedlings showed variation according to the bio-stimulant applied and were markedly distinct from those recorded in the untreated control. The results indicate that the bio-stimulant based on vermicompost combined with fermented plants induced

the highest flavonoid contents, followed by the fermented vermicompost formulation, and then by the plant-based bio-stimulants. In contrast, the control recorded the lowest flavonoid contents among all treatments evaluated (Figure 3.11).

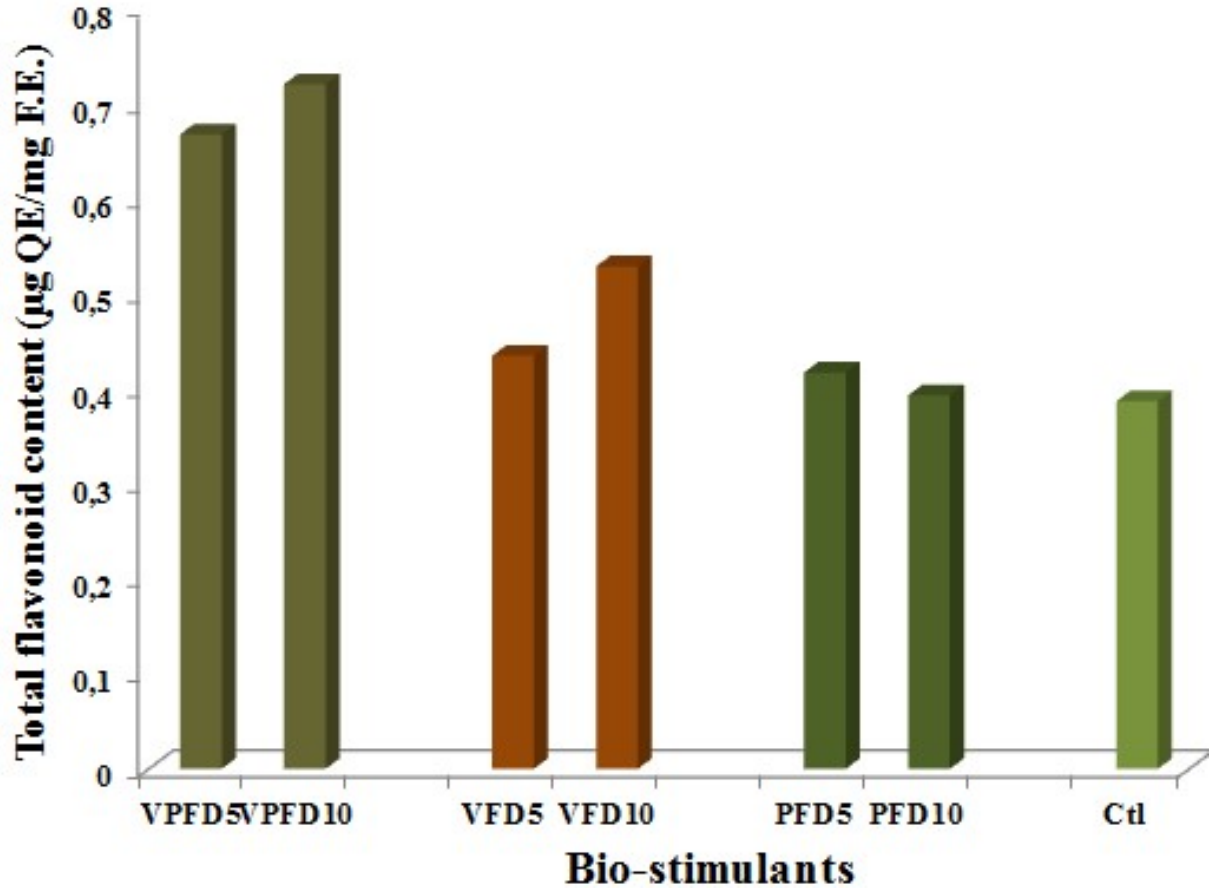


Figure 3.11: Effect of different dilutions of bio-stimulants on flavonoid concentration in tomato under saline stress. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants; Ctl: Control.

3.2 Comparison of the Microbial Load of the Different Fermented Substrates

Figure 3.12 illustrates the microbial activity recorded in the different types of bio-stimulants applied. The analysis of Log-CFU-per-g values (logarithm of the number of Colony-Forming Units per gram) shows that the highest microbial load was observed in treatments containing vermicompost. Indeed, the bio-stimulant based on fermented vermicompost exhibited a microbial load of 7.26 $\log_{10}$ CFU/g, followed by the treatment combining vermicompost with fermented plants, with 7.08 $\log_{10}$ CFU/g. In contrast, the bio-stimulant formulated solely from fermented plants recorded the lowest microbial load, with 5.13 $\log_{10}$ CFU/g.

These results highlight a significantly higher microbial activity in substrates enriched with vermicompost, suggesting that the latter constitutes a particularly favourable medium for the development and proliferation of microorganisms.

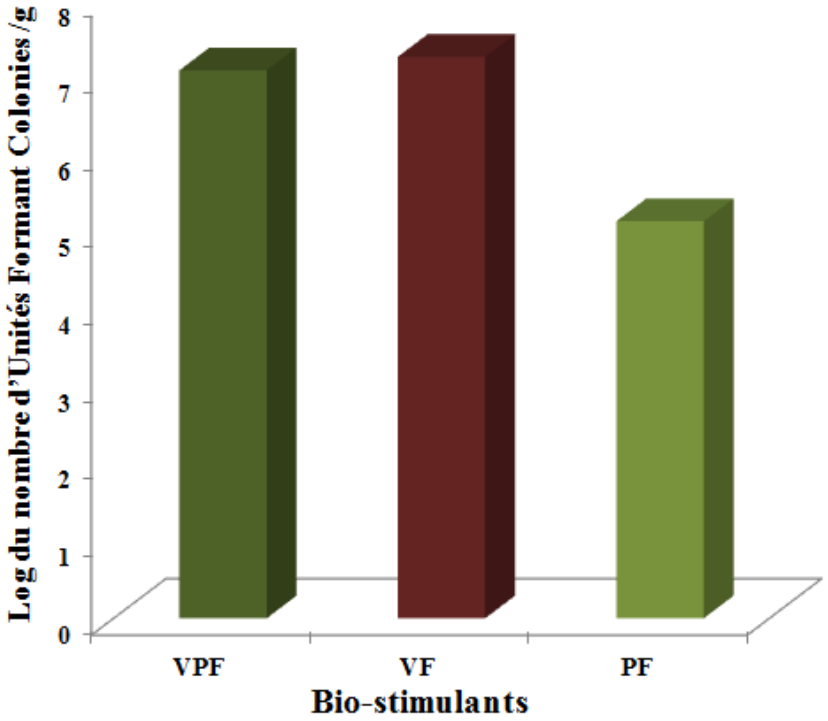


Figure 3.12: Comparative analysis of the microbial activity of the different bio-stimulants. VF: Fermented vermicompost; PF: Fermented plants; VPF: Vermicompost combined with fermented plants.

Chapter 4

DISCUSSION

General Discussion

Plants, being sessile organisms incapable of escaping unfavourable environments, are regularly exposed to adverse conditions that trigger specific or non-specific physiological responses. To cope with these constraints, they have developed two main categories of defence strategies: (i) stress avoidance, through morphological and biochemical barriers; and (ii) stress tolerance, via the activation of alternative metabolic pathways and preventive cellular mechanisms. In response to the damage caused by biotic and abiotic stresses, crop production and protection must build upon the stimulation of plant growth and development while minimising risks to human health and the environment, and ensuring the provision of safe, high-quality agricultural products [Posmyk and Szafrańska, 2016].

In this context, the use of biostimulants emerges as a promising approach, as it addresses the need for biological and ecological methods grounded in safe, active, and environmentally respectful substances.

The present study demonstrates the biostimulant effect of three distinct fermented preparations: a vermicompost tea (F1), a fermentation of aromatic and spontaneous plant species (*Eucalyptus* spp., *Cynodon dactylon*, *Urtica dioica*, *Rosmarinus officinalis*, *Inula* spp., and *Sinapis arvensis*) (F2), and a combined fermentation of vermicompost tea and these plant species (F3). Each preparation was applied as a liquid extract at two concentrations (5% and 10%) to evaluate their effects on growth parameters (cumulative aerial growth, root growth, aerial and root biomass, and leaf number), photosynthetic pigment contents (chlorophylls a, b, and total), phenolic and flavonoid compounds, and the accumulation of soluble osmolytes (proline and total sugars) in tomato plants (*Solanum lycopersicum* L., 1753) subjected to salt stress.

4.1 Effect of Fermented Formulations on the Growth Parameters of Salt-Stressed Tomato (*Solanum lycopersicum* L.)

Results of this study indicate that all biostimulant types applied exerted a significantly positive effect on the morphological and physio-biochemical parameters of the treated plants. These observations are consistent with previous research demonstrating that biostimulants operate across multiple levels of biological organisation — morphological, physiological, biochemical, epigenomic, proteomic, and transcriptomic — at each of which specific responses and metabolic modifications are induced, thereby contributing to the improvement of physiological performance and the adaptive capacity of plants facing biotic and abiotic environmental constraints [González-Morales et al., 2021].

The results reveal that the different dilutions (5% and 10%) of fermented vermicompost tea, aro-

matic plant fermentation, and their combined formulation all led to a progressive improvement across all assessed growth parameters, including cumulative aerial growth, root growth, aerial biomass, root biomass, and leaf number.

These findings suggest that the biostimulants constitute an important source of mineral elements and contain bioactive compounds, including phytohormones, enzymes, and other metabolites of interest, capable of stimulating plant metabolic processes and, as a consequence, promoting plant growth and development. This hypothesis is supported by several studies demonstrating that vermicompost tea exerts beneficial effects on the morphological and physiological parameters of plants. Multiple authors have confirmed that vermicompost tea can serve as an effective bio-fertiliser and biostimulant, providing a sustainable alternative to chemical fertilisers while improving plant growth and agricultural productivity [Katiyar et al., 2023].

Further evidence from Arancon et al. [2004] and Paneche et al. [2024] indicates that vermicompost results from the degradation of organic wastes through the joint action of earthworms and microorganisms under mesophilic conditions, yielding a humus-rich, stable organic material characterised by high concentrations of humic and fulvic acids, plant-available macro- and micronutrients (N, P, K, Ca, Mg, Fe), phytohormones such as indole-3-acetic acid, gibberellic acid, and cytokinins, as well as beneficial microorganisms. According to Visvanathan et al. [2005], only a small proportion of the organic matter ingested by earthworms — estimated at 5 to 10% — is actually assimilated, while the majority is excreted as fine granular aggregates associated with a mucus rich in organic compounds. These aggregates constitute an important source of nutrients, particularly nitrates, phosphates, and potassium, as well as micronutrients and soil- and plant-beneficial microorganisms.

Several authors have attributed the growth-promoting effect of vermicompost in tomato plants to its richness in growth-regulating substances of microbial origin, such as auxins, gibberellins, and cytokinins. Vermicomposts further contain a variety of bioactive components including antioxidants, phenolic compounds, phytohormones, humic and fulvic acids, and other organic acids [Guermache et al., 2018; Hosseinzadeh et al., 2015; Safiddine et al., 2019].

The results also show that the fermented vermicompost combined with aromatic and spontaneous plant species exerted the most pronounced biostimulant effect on tomato plants, compared to fermented vermicompost tea alone and then to the plant-only biostimulant formulation. These observations suggest that the combination of vermicompost tea with plant material may enhance the biostimulant properties of the final product by enriching it with bioactive compounds released during fermentation. In this regard, Lazcano et al. [2023] indicate that such a combination may generate a synergistic biostimulant effect superior to that obtained with either component applied separately.

Indeed, when plant materials are co-fermented with vermicompost, the resulting liquid product

combines the microbial richness and humic substances of the vermicompost with the phytochemical diversity of plant extracts. Degradation of plant tissues during fermentation promotes the extraction and solubilisation of numerous biologically active molecules, such as phenolic compounds, lipids, proteins, phytohormones, vitamins, and various organic and inorganic acids. These extracts may also contain essential macroelements, particularly nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), and magnesium (Mg), as well as several trace elements, including iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), molybdenum (Mo), and cobalt (Co). All of these compounds are likely to act synergistically with the humic substances and beneficial microorganisms derived from vermicompost, thereby enhancing the biostimulant potential of the formulation [Maignan, 2021]. The complexity of these formulations may thus explain their efficacy on plant development, notably through improved growth and stress tolerance, via actions at the cellular and molecular level on essential processes such as photosynthesis, metabolite synthesis, hormonal regulation, and enzymatic activity [Ali et al., 2020; Maignan, 2021].

The plant formulation investigated in this study includes several species recognised for their richness in bioactive compounds: *Eucalyptus* spp., rich in 1,8-cineole, tannins, and phenolic compounds with antioxidant and antimicrobial properties; *Urtica dioica* (stinging nettle), a source of flavonoids, chlorophyll, and mineral elements such as Fe, Ca, and Si capable of stimulating plant growth; *Rosmarinus officinalis* (rosemary), rich in rosmarinic acid, carnosic acid, and diterpenes; *Inula* spp. (fleabanes), associated with sesquiterpene lactones that may promote soil microbial activity; *Sinapis arvensis* (wild mustard), a source of glucosinolates; and *Cynodon dactylon* (Bermuda grass), containing various mineral elements and growth-regulatory substances [Plastira et al., 2024; Sadak et al., 2025].

Results further indicate that, following the saline stress period, treated plants exhibited less pronounced perturbation of growth parameters compared to the control, in which a marked deterioration — notably leaf fall — was observed. Leaves represent the most stress-sensitive aerial organs, particularly under abiotic stresses such as salinity. These observations suggest that plants adjust their energy allocation by reducing growth in order to redirect resources towards defence and adaptation mechanisms. Thus, the reduction in growth may be interpreted as an adaptive strategy enabling plant survival under salt stress [Zhu et al., 2012]. This growth inhibition may also be attributed to disturbances in the levels of growth regulators in plant tissues, particularly abscisic acid and cytokinins, whose balance is strongly affected by the presence of salt [Fahad et al., 2015].

4.2 Effect of Fermented Formulations on the Physio-Biochemical Parameters of Salt-Stressed Tomato (*Solanum lycopersicum* L.)

Results indicate that the different formulations, and particularly the plant-based fermented tea, exerted a significant effect on chlorophyll synthesis compared to the control. These observations suggest that vermicompost tea combined with plant species is rich in mineral elements and organic compounds, thereby promoting enhanced photosynthetic activity. This hypothesis is supported by the work of Guermache et al. [2018], who demonstrated that vermicompost application contributes to increased chlorophyll and carotenoid contents in leaves, thereby improving the photosynthetic efficiency of plants.

However, a significant decrease in total chlorophyll content was observed following the imposition of salt stress, although this decline was less pronounced in treated plants compared to the control. It was found that chlorophyll content decreased with increasing salt stress intensity, in accordance with the work of several authors demonstrating that NaCl exerts a strongly negative effect on leaf chlorophyll content. Indeed, the presence of NaCl in the culture medium leads to chlorophyll reduction from concentrations as low as 7.5 g L^{-1} [Kaab and Kardou, 2024]. Moreover, Mesquita et al. [2007] also demonstrated that cashew nut seedling photosynthesis is strongly affected by salinity.

Results show that proline accumulation was higher in plants treated with biostimulants, both before and after stress application, compared to the control. Several studies have previously reported increased proline contents in response to salt stress [Kaab and Kardou, 2024].

Ashraf and Foolad [2007b] also demonstrated that, under salt stress conditions, many plant species accumulate proline to protect cells against the deleterious effects of salt. This accumulation contributes to the maintenance of osmotic balance and to the protection and stabilisation of cellular structures. Accordingly, proline accumulation represents one of the primary adaptive strategies employed by plants to mitigate the effects of various types of abiotic stress.

Results indicate that the accumulation of total soluble sugars was higher in plants treated with biostimulants compared to the control. These findings suggest that such sugar accumulation may be linked to the activation of basal plant metabolism. Similar results have been reported in other plant species treated with composted amendments [Chaichi and Djazouli, 2017]. Furthermore, Munns and Tester [2008] demonstrated that saline stress induces the accumulation of soluble sugars — such as glucose, fructose, and sucrose — in plant tissues. These compounds play an essential role in osmoregulation as well as in the stabilisation of cell membranes and proteins.

In the present study, the application of biostimulants resulted in a slight increase in total phenolic

compound content in tomato plants subjected to salt stress, compared to the untreated control. These findings are consistent with those reported by [Benazzouk et al., 2020], who demonstrated that phenolic compounds play a significant role in plant response and adaptation mechanisms to various environmental stresses, owing to their potent antioxidant capacity.

Furthermore, vermicompost is widely recognized for its richness in bioactive compounds, particularly salicylic acid (SA) and benzoic acid (BzA). These two phenolic compounds, endowed with hormonal activity, are involved in the regulation of plant metabolism and play an essential role in growth, development, and defense mechanisms of plants. They also constitute an important source of antioxidants, contributing to the protection of plant cells and their constituents against oxidative damage induced by salt stress [El-Shora and Soror, 2024].

Chapter 5

Presentation and Economic Study of the Startup Project

Introduction

Plants, owing to their inability to move, are continuously exposed to numerous stress factors present in their environment. These constraints may be of biotic origin (pathogenic agents, insect pests) or abiotic origin (salt, water, thermal, and chemical stress, among others), and directly affect plant growth, development, and yield.

Among the various types of abiotic stress, salinity constitutes one of the major challenges of modern agriculture. The excessive accumulation of salts in the soil disrupts the absorption of water and nutrients by the roots, causing a physiological imbalance that limits plant growth and significantly reduces crop productivity.

To cope with these unfavourable conditions, plants have developed, throughout the course of their evolution, various adaptive mechanisms enabling them to maintain their physiological balance and survival. In this perspective, the use of natural bio-stimulants is generating growing interest in sustainable agriculture. These products promote nutrient absorption, stimulate the physiological processes of plants, and enhance their capacity to tolerate environmental stresses, while reducing the negative impact of conventional agricultural practices on the environment and human health.

It is within this context that the present startup project is situated. The project aims to develop an innovative, natural, and environmentally friendly bio-stimulant obtained from the natural fermentation of a mixture of vermicompost and aromatic plants. This solution valorises local biological resources and is fully aligned with the principles of sustainable and reasoned agriculture.

The objective is to provide farmers with an ecological alternative to chemical fertilisers and inputs, capable of improving crop growth, strengthening plant resistance to biotic and abiotic stresses — particularly salt stress — and contributing to a more productive and environmentally responsible agricultural output. Through this innovation, the ambition is to participate in the transition towards greener agriculture, reconciling productivity, biodiversity preservation, and food security.

Beyond its agronomic interest, this innovation contributes to reducing dependence on chemical inputs, preserving soil fertility, and promoting a healthier and more sustainable agricultural production. It thus represents an alternative that is ecologically sound, economically accessible, and scientifically validated, capable of accompanying the transition towards more resilient, productive, and environmentally adapted production systems.

5.1 Work Team and Supervision

5.1.1 Project Team

The project team is composed of the following member:

Table 5.1: Project team composition

Team Member	Speciality	Faculty / Institute	Experience and Competence
Student 1: SABOUR Bochra	Phytopathology	Department of Agronomy, Faculty of Sciences, University of Médéa	Bachelor's degree in Plant Protection

5.1.2 Supervision Team

The supervision team is composed of the following members:

Table 5.2: Supervision team composition

Supervisor	Speciality	Faculty / Institute
Supervisor: TCHAKER Fatma Zahra	Agricultural Sciences — Plant Protection and Environment	Department of Agronomy, Faculty of Sciences, University of Médéa
Co-Supervisor: SAFIDDINE Fadhila	Agricultural Sciences — Bio-pesticides	—

5.2 Project Description

5.2.1 Definition of the Activity

The present project consists of developing and producing a natural, environmentally friendly biostimulant based on the use of organic matter and biological resources. This product is designed to stimulate plant growth as well as plant defence mechanisms against various types of stress, whether biotic or abiotic.

The activity is based on the valorisation of a process involving the natural fermentation of vermicompost, the fermentation of aromatic and spontaneous plants (*Eucalyptus*, couch grass, nettle, rosemary, inula, and field mustard), as well as the fermentation of a mixture combining vermicompost and aromatic and spontaneous plants. This fermentation process yields a biological product rich in active compounds, macro- and micro-elements, and beneficial microorganisms.

These bio-stimulants promote nutrient absorption, stimulate soil biological activity, improve the physiological performance of crops, and strengthen their resilience under adverse environmental conditions, thereby contributing to optimising plant growth while maintaining the balance of agricultural ecosystems.

This project is part of a sustainable and innovative agricultural approach, proposing a natural, effective, and efficient alternative to chemical fertilisers and inputs. It aims to accompany farmers towards more ecological practices, improving crop productivity while reducing negative impacts on the environment and human health.

5.2.2 Sector of Activity

The project falls within the following sectors of activity:

- **Industry:** production of a bio-stimulant based on fermented vermicompost enriched with aromatic plants.
- **Agriculture:** particularly in the fields of agricultural biotechnology and organic farming.

Furthermore, this project belongs to the category of agro-ecological and innovative projects, aiming to promote green, sustainable, and environmentally responsible agriculture.

5.2.3 Activity Code

- **Code: 201299** — Other manufacturing activities of fertilisers and nitrogen compounds.
- **Description:** Activities related to the production of products intended to stimulate plant growth and strengthen plant defence mechanisms under adverse conditions, in order to improve plant development and ensure better agricultural yield.
- **Justification:** The startup project consists of producing natural bio-stimulants intended to improve plant performance. This activity falls directly within the domain of integrated crop production and protection, offering alternative methods to chemical fertilisers, inputs, and stimulants.

5.3 Description of the Project Concept

5.3.1 Project Products

The proposed product is a natural bio-stimulant named **VermiHerb Shield**, designed to improve plant performance and resilience against various environmental constraints. This bio-stimulant is produced from fermented organic resources in an aqueous medium over a period ranging

from 20 days to one month. It is primarily composed of vermicompost — derived from the joint action of earthworms and associated microorganisms on organic matter at ambient temperature — as well as aromatic and spontaneous plants, namely *Eucalyptus*, couch grass, nettle, rosemary, inula, and field mustard.

In order to optimise their stimulatory potential, these raw materials are subjected to a controlled aqueous fermentation process, promoting the release of bioactive compounds, macro- and micro-elements, as well as the development of significant beneficial microbial activity.

The resulting product constitutes an effective biological solution that contributes to reducing dependence on costly chemical inputs, while promoting the sustainable management of agricultural production systems.

5.3.2 Target Customers

The market for this natural product encompasses a wide range of stakeholders concerned with improving agricultural production.

Public Sector

This segment represents a strategic actor in the adoption and dissemination of innovative agricultural solutions. It includes:

- The Ministry of Agriculture and Rural Development, as well as its various directorates;
- Specialised technical institutes, such as the National Institute of Plant Protection (INPV), the Technical Institute of Field Crops (ITGC), the Technical Institute for Vegetable and Industrial Crops (ITCMI), and the Technical Institute for Fruit Arboriculture and Viticulture (ITAFV);
- Agricultural research centres and public bodies responsible for the experimentation and dissemination of agricultural innovations;
- The Ministry of Commerce and Export Promotion, along with all its directorates and sectors.

Private Sector

This segment constitutes the core of direct product use. It includes:

- Farmers;
- Agricultural holdings and cooperatives;
- Pilot farms and demonstration holdings;

- Private agricultural enterprises engaged in organic farming;
- Plant protection product companies.

The product is thus positioned within a logic of complementarity between the public and private sectors, with the objective of progressively promoting the adoption of natural solutions in modern agricultural practices.

Justification of Sector Selection

The selection of these sectors is based on their strategic role in the development, valorisation, and dissemination of innovative agricultural products. The public sector, through ministries and agricultural institutions, constitutes a key actor due to its constant need for bio-stimulants aimed at improving and ensuring agricultural productivity at the national level. Scientific research centres also play an essential role in knowledge development and the validation of new products. Their sensitivity to the side effects of chemical inputs reinforces their potential for adopting ecological solutions. The Ministry of Commerce contributes to market structuring by ensuring the expansion of the distribution network and the availability of bio-stimulants throughout the national territory.

The private sector encompasses the principal users, notably farmers and agricultural holdings, characterised by a constant need for bio-fertiliser and bio-stimulant solutions for crops. This segmentation thus allows for the effective targeting of stakeholders most likely to adopt alternatives to chemical inputs, within the framework of sustainable and environmentally responsible agriculture.

5.3.3 Project Location and Distribution

Project Location

The project will be carried out in a laboratory equipped with the necessary tools and equipment, where the stages of vermicompost production, aqueous fermentation, as well as tests related to plant growth stimulation and plant defence against various types of stress will be conducted.

The project will preferably be established in an agricultural region, such as the wilaya of Médéa, in order to facilitate direct access to the raw materials necessary for the manufacture of bio-stimulants, notably the collection of organic waste for vermicompost production and the harvesting of aromatic and spontaneous plants used in the fermentation process. This location also ensures proximity to the main product users, thereby facilitating field trials and the dissemination of bio-stimulants.

Product Distribution

Several distribution channels may be selected:

- **Direct distribution:** selling the product directly from the manufacturer to the customer, without intermediaries, including through the company's own points of sale or e-commerce websites.
- **Indirect distribution:** using intermediaries or distributors to deliver the product to customers, including retailers, wholesalers, and distribution agents.
- **Multi-channel distribution:** using both distribution channels simultaneously to meet the diverse needs and preferences of customers.

Promotion

In order to ensure better product visibility and strengthen its market presence, the following communication and promotion actions will be implemented:

- **Advertising:** printed and online advertisements to disseminate information about the product, its components, mode of use, and agricultural importance.
- **Sales promotions:** discounts and special offers to reinforce the product's perceived value and encourage purchase.
- **Media relations:** establishing solid media relationships to increase product awareness, including participation in agricultural fairs and exhibitions.

5.3.4 Project Operations

Production constitutes a key stage in the success of the project. Its implementation requires a qualified technical team and compliance with the standards in force in agro-industrial activities. The production process is based on the following complementary stages:

- **Selection of earthworm species:** The species *Eisenia fetida* (Savigny, 1826) is selected for vermicompost production, owing to its high efficiency in organic matter degradation;
- **Selection of raw materials:** Organic materials, notably organic waste and aromatic and spontaneous plants, are carefully selected and collected;
- **Preparation of bio-stimulants:** The vermicompost and selected plants are subjected to aqueous fermentation processes to obtain a bio-stimulant rich in bioactive compounds and mineral elements;
- **Quality control:** The resulting products are analysed, tested, and optimised to ensure their efficacy and conformity prior to any scale-up in production;

- **Conditioning and packaging:** The product is packaged in sterile, hermetically sealed plastic containers, then labelled with the necessary information concerning its use;
- **Storage:** The product is kept under appropriate conditions to preserve its stability, quality, and efficacy until commercialisation;
- **Commercialisation and distribution:** The product is distributed through various commercial circuits, primarily targeting the agricultural sector.

Technical support may be offered to users to ensure optimal product use and guarantee its effectiveness in the field.

5.3.5 Justification of the Project Concept

The idea for this project arose from the growing increase in environmental constraints, primarily due to anthropogenic activities, which negatively affect plant production systems. These disturbances result notably in soil degradation, a decline in soil fertility, and an increase in abiotic stresses to which plants are exposed.

In this context, it becomes necessary to develop sustainable alternatives to conventional chemical inputs in order to strengthen plant tolerance and improve plant performance under these constraints, while ensuring a stable and resilient agricultural productivity. This approach relies on the valorisation of biodegradable, available, low-cost, and environmentally neutral resources, acting on plant growth stimulation, improvement of soil biological activity, and reinforcement of natural plant defence mechanisms against environmental stresses.

This solution enables the following:

- Reducing dependence on costly and polluting chemical inputs;
- Valorising organic waste and local natural resources;
- Improving soil biological fertility;
- Strengthening crop resilience against environmental constraints;
- Offering an accessible and economically viable solution for farmers.

5.4 Market Study

5.4.1 Nature and Intensity of Competition

In the context of this bio-stimulant project, the main competitors are companies specialising in chemical agricultural inputs, as well as companies offering biological control and bio-stimulation

solutions already present on the market.

Table 5.3: Analysis of competitive landscape

Competitors	Nature		Intensity		
	Direct	Indirect	High	Medium	Low
Manufacturers and importers of chemical products (fertilisers, pesticides, etc.)	X		X		
Manufacturers and importers of biological products (bio-fertilisers, bio-stimulants)		X			X

In response to this competition, the company adopts several complementary strategies:

- **Differentiation strategy:** proposing a 100% bio-ecological, natural, and environmentally responsible solution that respects biodiversity and human health;
- **Cost leadership strategy:** using biodegradable organic raw materials to offer a product with reduced production costs and a competitive market price;
- **Innovation strategy:** developing bio-stimulants based on vermicompost and aromatic and spontaneous plants, optimised through natural fermentation processes;
- **Farmer awareness strategy:** informing farmers of the negative impacts of chemical fertilisers and the advantages of biological solutions;
- **Customer proximity strategy:** providing technical support and field monitoring during initial use to guarantee product efficacy and strengthen user confidence.

5.4.2 Target Market Size and Potential

Target Market Size

The target market is composed of actors in the agricultural sector, both public and private, representing a strategic domain of the economy. It encompasses public institutions (Ministry of Agriculture and Rural Development, technical institutes, and research centres) as well as private actors, notably farmers, agricultural holdings and cooperatives, pilot farms, organic farming enterprises, and plant protection stakeholders. These customers are primarily located in regions with strong agricultural vocation, distributed across the national territory, with a particular concentration in the wilaya of Médéa.

The size of this market is considerable, given the growing demand for effective solutions to improve plant tolerance to stress factors responsible for significant agricultural losses. Furthermore, the progressive awareness among farmers regarding the environmental and health impacts of chemical products reinforces the demand for sustainable, natural, and economically accessi-

ble alternatives.

Potential Market Size

The potential market encompasses all users likely to adopt alternative solutions to chemical products for improving agricultural yield. It includes the customers of the target market, as well as rural development bodies, forestry establishments, national parks, nature reserves, and any structure involved in environmental protection and sustainable ecosystem management. This market presents a strong growth potential, driven by increasing demand for biological, ecological, and sustainable solutions in the agricultural and environmental sectors.

5.4.3 SWOT Analysis of the Project

5.4.3.1 Factors Favouring the Achievement of Objectives

Strengths

- Innovation based on the use of fermented vermicompost to stimulate plant growth and strengthen plant defences;
- Simple, accessible, and easily reproducible manufacturing process;
- Valorisation of local, low-cost raw materials, contributing to cost reduction and circular economy;
- Competitive price compared to products available on the market;
- Ecological and biodegradable solution, consistent with environmental objectives aimed at reducing chemical inputs;
- Compatibility with organic farming and the principles of integrated crop protection;
- Safe product, owing to the absence or very low content of toxic substances;
- Local production favouring job creation and regional economic development;
- Competent project team with scientific knowledge in bio-control and integrated crop production.

Opportunities

- Growing demand for biological products and sustainable solutions for crop protection and stimulation;
- Strengthening of public policies in favour of ecological transition and environmental protection;

- State support within the framework of national mechanisms (notably Order 1275) encouraging innovative and university-based projects;
- Existence of support programmes for innovation and agricultural entrepreneurship;
- Opportunities for partnerships with research institutes, agricultural cooperatives, and rural development bodies.

5.4.3.2 Factors Impeding the Achievement of Objectives

Weaknesses

- Need for significant quantities of organic raw materials to ensure production;
- Seasonal availability of certain plants, which may affect manufacturing regularity;
- Relatively limited shelf life, requiring appropriate storage conditions;
- Variable efficacy depending on cultivation conditions, soil type, and plant species;
- Possible resistance from some farmers to adopting this technology due to lack of information;
- Absence of strong initial product recognition on the market;
- Need for technical monitoring during initial use to optimise results;
- Dependence on biological processes sensitive to environmental conditions;
- Potential difficulty in standardising the product at scale due to its biological nature.

Threats

- Strong competition from chemical inputs already established on the market;
- Resistance to change among farmers accustomed to chemical treatments;
- Risk of emergence of competing technologies with superior performance.

5.4.4 Business Model Canvas

The Business Model Canvas (BMC) allows for the structured and synthetic presentation of the overall functioning of the project, highlighting the essential elements related to value creation, customers, resources, and revenue streams (Table 5.4).

Table 5.4: Business Model Canvas of the project

<table>
<tr><th>Key Partners</th><th>Key Activities</th><th>Value Proposition</th><th>Customer Relationships</th><th>Customers</th></tr>
<tr><td>Ministry of Agriculture; Agricultural directorates and technical institutes; Agricultural research centres; Agricultural cooperatives and pilot farms; Local organic material suppliers</td><td>Selection and collection of raw materials; Vermicompost production and bio-stimulant elaboration; Agronomic efficacy testing; Quality control; Conditioning and logistics; Marketing and farmer awareness; Technical support</td><td>Bio-ecological and biodegradable solution; Reduction of chemical input use; Low-cost accessible product; Sustainable crop production; Respect for biodiversity and human health; Absence of toxic residues</td><td>Technical farmer support; Usage awareness and advice; Field performance monitoring; After-sales assistance</td><td>Farmers; Cooperatives and holdings; Pilot and demonstration farms; Agricultural public institutions</td></tr>
<tr><td rowspan="2"></td><td>Key Resources</td><td rowspan="2"></td><td>Distribution Channels</td><td rowspan="2"></td></tr>
<tr><td>R&D team; Laboratory and production equipment; Local organic raw materials; Scientific knowledge in biological attractants</td><td>Direct sales; Indirect sales via intermediaries; Digital platform for orders and advice</td></tr>
<tr><td colspan="2">Cost Structure: Purchase of raw materials; Laboratory equipment; R&D testing; Packaging; Transport; Qualified labour</td><td colspan="3">Revenue Streams: Sale of fermented vermicompost; Institutional partnerships; Grants and subsidies; Technical consultancy services</td></tr>
</table>

5.5 Technical Study

5.5.1 Human Resource Requirements

The proper functioning of the project requires a team with scientific, technical, administrative, and commercial competencies. The human resource requirements are presented in Table 5.5.

Table 5.5: Distribution of human resources required for project operations

Position	Number	Qualifications / Diplomas
Manager / Project Manager	01	Master's degree in Plant Protection or Bio-control
Technical Staff	01	Master's degree in Plant Protection, Bio-control, or Higher Technician in Agriculture
Operational Staff (Laboratory and Production)	01	Agronomist engineer, biologist, or higher laboratory technician
Control Staff (Stock Management)	01	Bachelor's degree in Management, Logistics, or Commerce
Commercial Officer	01	Bachelor's or Master's degree in Commerce, Marketing, or Business Administration
Support Staff	01	Diploma in Management, Administration, or Secretarial Studies
Operational Staff (Driver-Delivery, Cleaning)	02	Appropriate driving licence; experience in transport and delivery
Maintenance and Security Agent	01	Secondary level or vocational training in hygiene, maintenance, or security

5.5.2 Project Location

The project will preferably be established in a region with a strong agricultural vocation, while remaining close to an urban centre to facilitate production, supply, commercialisation, and distribution activities. The wilaya of Médéa constitutes an adapted strategic site, with the following justifications:

- **Proximity to raw materials:** facilitated access to aromatic and spontaneous plants, organic resources, and bio-waste for vermicompost preparation;
- **Proximity to the target market:** the wilaya is characterised by significant agricultural activity in arboriculture, olive growing, and field crops, ensuring direct contact with potential users;
- **Ease of commercialisation:** proximity to urban centres and main road axes facilitates product delivery and reduces logistical costs;
- **Field experimentation opportunities:** the presence of numerous agricultural holdings allows for trials and product evaluations under real conditions;
- **Availability of labour and partners:** the region offers access to technical skills and potential partners (cooperatives, technical institutes, development bodies).

5.6 Cost and Revenue Estimation

5.6.1 Estimation of Total Investment

The estimation of all initial expenditures required for the realisation of the project is presented in Table 5.6.

Table 5.6: Estimation of project investment costs

Item	**Amount (DA)**
Production machinery and equipment (vermicomposting unit, rearing chamber, grinder, fermenters, balances, pH-meter, thermometer, moisture meter, binocular magnifier, distiller, glassware, etc.)	1,200,000.00
Premises development	1,000,000.00
Rolling stock (transport vehicles)	2,500,000.00
Office furniture and equipment	200,000.00
Computer equipment	200,000.00
Audio-visual equipment	100,000.00
Communication equipment	80,000.00
Equipment installation	200,000.00
General incorporation expenses	200,000.00
Working capital (initial raw materials)	300,000.00
Total Investment (Project Cost)	**5,980,000.00 DA**

5.6.2 Estimation of Raw Material Purchases

The estimation of raw material requirements over a three-year period is summarised in Table 5.7.

Table 5.7: Estimation of raw material purchases over three years

Raw Material	Item	Year 1	Year 2	Year 3
Material 1: Earthworm *Eisenia fetida* (Savigny, 1826)	Quantity (ind.)	10,000	15,000	20,000
	Unit price (DA/ind.)	10	10	10
	Cost (DA)	**100,000**	**150,000**	**200,000**
Material 2: Organic waste	Quantity (kg)	600	800	1,000
	Unit price (DA/kg)	10	10	10
	Cost (DA)	**6,000**	**8,000**	**10,000**
Material 3: Aromatic plants	Quantity (kg)	1,500	3,000	4,000
	Unit price (DA/kg)	20	20	20
	Cost (DA)	**30,000**	**60,000**	**80,000**
Material 4: Coconut fibre	Quantity (units)	10	15	20
	Unit price (DA/unit)	1,000	1,000	1,000
	Cost (DA)	**10,000**	**15,000**	**20,000**
Material 5: 2L bottles (packaging)	Quantity (units)	10,000	15,000	25,000
	Unit price (DA/unit)	20	20	20
	Cost (DA)	**200,000**	**300,000**	**500,000**
Material 6: Packaging and labels	Quantity (units)	10,000	15,000	25,000
	Unit price (DA/unit)	10	10	10
	Cost (DA)	**100,000**	**150,000**	**250,000**
Total Raw Material Purchases (DA)		**446,000**	**683,000**	**1,060,000**

5.6.3 Estimation of Labour Costs

The estimation of the wage bill over the first three years is presented in Table 5.8.

Table 5.8: Estimation of staffing levels and wage bill over three years

Position	Number			Monthly Salary (DA)		
	Y1	Y2	Y3	Y1	Y2	Y3
Manager / Project Manager	01	01	01	55,000	55,000	60,000
Technical Staff	01	01	01	50,000	52,000	55,000
Operational Staff (Lab. & Production)	01	02	02	45,000	48,000	50,000
Control Staff (Stock Management)	01	01	01	35,000	37,000	40,000
Commercial Officer	01	01	01	40,000	42,000	44,000
Support Staff	01	01	01	32,000	34,000	36,000
Driver-Delivery Agent	01	01	01	30,000	32,000	34,000
Cleaning Agent	01	01	01	25,000	27,000	30,000
Maintenance and Security Agent	01	01	01	25,000	27,000	30,000
Total (annual / 12 months)	**09**	**10**	**10**	**4,044,000**	**4,248,000**	**4,548,000**

5.6.4 Estimation of External Operating Charges

External charges encompass all expenditures necessary for the operation of the project that are not directly linked to production (Table 5.9).

Table 5.9: Estimation of external operating charges (DA)

Item	Year 1	Year 2	Year 3
Electricity, gas, and water expenses	120,000	140,000	160,000
Maintenance and repair expenses	100,000	120,000	160,000
Premises rental expenses	360,000	360,000	360,000
Equipment and machinery insurance	40,000	40,000	40,000
Marketing expenses (advertising and promotion)	100,000	150,000	200,000
Training and professional development expenses	50,000	50,000	50,000
Other general external charges	60,000	80,000	100,000
Total	**830,000**	**940,000**	**1,070,000**

5.6.5 Estimation of Investment Depreciation

The estimation of annual depreciation of investments is summarised in Table 5.10.

Table 5.10: Estimation of annual depreciation of investments

Item	Period (years)	Annual Depreciation (DA)
Production machinery and equipment	10	120,000
Transport equipment	05	500,000
Office furniture and equipment	10	20,000
Computer equipment	05	40,000
Audio-visual equipment	05	20,000
Communication equipment	05	16,000
Total	—	**716,000 DA**

5.6.6 Revenue Estimation

The planned sales quantities, unit prices, and total sales values are presented in Table 5.11.

Table 5.11: Estimation of annual project turnover

Products Sold	Item	Year 1	Year 2	Year 3
Product 1: Bio-stimulant (2L bottles)	Quantity sold	10,000	15,000	25,000
	Unit price (DA)	1,200	1,200	1,200
	Sales value (DA)	**12,000,000**	**18,000,000**	**30,000,000**
Service 2: Technical advice and monitoring	Quantity	10	15	20
	Unit price (DA)	10,000	10,000	10,000
	Sales value (DA)	**100,000**	**150,000**	**200,000**
Total Amount (DA)		**12,100,000**	**18,150,000**	**30,200,000**

5.6.7 Annual Project Result

Table 5.12 presents the estimation of the projected annual result over a three-year period, highlighting the evolution of turnover, operating charges, depreciation, and the expected net result,

thereby enabling the evaluation of the project's profitability and economic viability over the medium term.

Table 5.12: Estimation of projected annual project result

Item	Year 1	Year 2	Year 3
Turnover (1)	12,100,000	18,150,000	30,200,000
Raw materials consumed (2)	446,000	683,000	1,060,000
External charges (3)	830,000	940,000	1,070,000
Value Added (4) = (1)–(2)–(3)	**10,824,000**	**16,527,000**	**28,070,000**
Salaries and social charges (5)	4,044,000	4,248,000	4,548,000
Gross Operating Surplus (6) = (4)–(5)	**6,780,000**	**12,279,000**	**23,522,000**
Depreciation allowances (7)	716,000	716,000	716,000
Operating Result (8) = (6)–(7)	**6,064,000**	**11,563,000**	**22,806,000**
Tax (10%) (9)	606,400	1,156,300	2,280,600
Net Result (10) = (8)–(9)	**5,457,600**	**10,406,700**	**20,525,400**
Turnover growth rate	—	50%	66.39%

5.7 Conclusion

This project is part of a dynamic transition towards sustainable, innovative, and environmentally responsible agriculture. It provides a response to current challenges related to the reduction of chemical input use, by proposing a bio-stimulation and bio-control solution adapted to the needs of the agricultural sector.

From a scientific perspective, it valorises the results of university research by transforming them into an innovative and field-applicable solution. From a technological and environmental perspective, it proposes an ecological alternative based on the valorisation of local organic resources, thereby contributing to the preservation of soils and ecosystems. From an economic perspective, the project reduces dependence on imported inputs, lowers production costs, and generates local added value. From a social perspective, it promotes job creation and the capacity building of farmers through the dissemination of sustainable agricultural practices.

Owing to its innovative, ecological, and economically viable character, this project presents a strong development potential and contributes to the improvement of agricultural productivity as well as food security.

General Conclusion

The present study has highlighted the potential of fermented biostimulants in improving tomato tolerance to salt stress. The results obtained demonstrate that the application of the various tested formulations had an overall favorable effect on the morphological, physiological, and biochemical parameters of tomato plants, compared to the control. In particular, the biostimulants contributed to stimulating vegetative growth, improving photosynthetic pigment contents as well as phenolic and flavonoid compound levels, reflecting an enhanced adaptive capacity of the plants under salinity conditions.

Among the treatments studied, the formulation combining vermicompost with fermented aromatic plants distinguished itself by its superior efficacy, followed by fermented vermicompost and then fermented plants. This performance could be attributed to the richness of vermicompost in nutrient elements, bioactive substances, and beneficial microorganisms, reinforced by the contribution of metabolites derived from fermented aromatic plants. The greater microbial activity observed in formulations containing vermicompost also appears to constitute a key factor in improving the responses of plants subjected to salt stress.

Overall, this study confirms the potential of fermented biostimulants as an innovative biological solution to mitigate the effects of salt stress and promote more sustainable agriculture. The valorization of vermicompost and local plant resources through fermentation processes thus appears as a promising pathway for developing bioecological alternatives to conventional inputs.

In light of the results obtained, it would be worthwhile to further this study by:

- Utilizing the formulated biostimulant within the framework of an integrated pest management program;
- Evaluating certain physiological parameters, phytohormones, and mineral elements in order to better understand the application mode and optimal conditions of the different molecules;
- Characterizing more precisely the microbial populations and bioactive compounds involved in the biostimulant effect;
- Validating the efficacy of these formulations under field conditions in order to confirm their agronomic potential in real production systems;
- Studying their long-term impact on soil fertility, mineral nutrition, and crop productivity.

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