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Master's Thesis

**Exploring functionalized biopolymer prototypes towards
mitigation of salt stress in strawberry plants**

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Limassol, May 2026

CYPRUS UNIVERSITY OF TECHNOLOGY
FACULTY OF GEOTECHNICAL SCIENCES AND
ENVIRONMENTAL MANAGEMENT
DEPARTMENT OF AGRICUTLTURAL SCIENCES,
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ABSTRACT

Soil salinity is one of the major abiotic stresses limiting crop productivity worldwide, particularly in arid and semi-arid regions. Present study explored the potential of salicylic acid (SA) encapsulated in zein and sodium alginate functionalized with graphene oxide (GO) as innovative priming agents to mitigate salinity stress in strawberry (*Fragaria × ananassa*) plants. The experiment was conducted between April and June 2025 where fresh rooted strawberry plants were planted in 1.4L pots in peat-perlite substrate (3:2 ratio) under controlled conditions and fertilized with a mixture of minerals. Different concentrations of salicylic acid (SA), zein, and their encapsulated formulations, as well as graphene oxide (GO) and GO-functionalized sodium alginate (NaA–GO), were evaluated to assess their effectiveness against salinity stress. Three foliar treatment applications were performed at different time-points prior to flowering, followed by salinity stress initiation (100 mM NaCl) 2 days after the last priming application. Physiological and biochemical parameters were evaluated to assess plant performance and oxidative stress responses in response to the different treatments. Physiological measurements and leaf sampling were performed at 2-, 4-, 6- and 8-days post-treatment under salinity stress. Biochemical markers including malondialdehyde (MDA), hydrogen peroxide (H₂O₂), proline, and nitric oxide (NO) were quantified. Results demonstrated that all treatments effectively mitigate the negative impacts of salinity stress on strawberry plants. At early stages of stress induction, Zein and SA alone significantly reduce MDA content while encapsulated formulations showed consistent reductions in MDA content across all time points, with 50 mg/L Zein–SA exhibiting the strongest effect. Similarly, GO and GO-functionalized sodium alginate treatments significantly decreased MDA levels while hydrogen peroxide content was more variably affected. However, encapsulated formulations and GO treatments at 0.02–0.04 mg/mL markedly reduced H₂O₂ accumulation, suggesting effective ROS detoxification. Proline content was significantly

reduced in all SA, Zein and encapsulated treatments reflecting the strong treatment impact on osmotic balance. Functionalized NaA–GO combinations displayed strong performance in decreasing proline content across all time points, confirming their role in enhancing stress tolerance. Nitric oxide levels also decreased significantly, particularly under intermediate concentrations of SA and GO. Encapsulation and nanocarrier systems provide a promising approach for the mitigation of salinity-induced oxidative stress. The observed reductions in biochemical stress markers, suggest that these treatments can effectively limit oxidative damage under abiotic stress conditions. The study highlights the synergistic effect of bioactive compounds with nanocarrier systems in the mitigation of oxidative damage induced under saline environments.

Keywords: *Salinity stress; Strawberry (Fragaria × ananassa); Salicylic acid; Zein nanoparticles; Graphene oxide; Sodium alginate; Nanoencapsulation; Priming agents; Oxidative stress; Reactive oxygen species (ROS); Abiotic stress tolerance; Nanocarrier systems; Malondialdehyde (MDA); Hydrogen peroxide (H₂O₂); Proline accumulation; Nitric oxide (NO); Foliar application; Stress mitigation; Plant nanotechnology; Saline environments.*

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1 INTRODUCTION

Soil salinity represents one of the most serious environmental constraints limiting agricultural productivity worldwide, particularly in arid and semi-arid regions. Approximately 1.0 billion hectares of agricultural land are affected by salinity, while nearly 100 million hectares of arable land are estimated to suffer from salt accumulation and its adverse effects on crop production (Karlidag et al., 2009). Salinity exerts detrimental effects on plant growth and development through the induction of osmotic imbalance, ion toxicity and oxidative stress. These processes lead to excessive generation of reactive oxygen species (ROS), which can damage cellular components and disrupt normal physiological functions (Li et al., 2022). Among horticultural crops, strawberry (*Fragaria × ananassa*) is considered highly sensitive to salt stress, making its cultivation particularly vulnerable in saline environments. Previous studies have demonstrated that salinity can significantly reduce leaf expansion, shoot biomass, crown branching, and ultimately fruit yield in strawberry plants (Abu Zeid et al., 2023). Despite these limitations, the global cultivation of strawberry continues to expand, increasing the need to address its susceptibility to saline conditions (Sun et al., 2015). Therefore, the development of strategies aimed at enhancing salt tolerance in strawberry is essential for maintaining sustainable production and ensuring crop performance under increasing salinity stress.

1.1 Strawberry production and salinity stress

Strawberry (*Fragaria × ananassa* Duch.) is one of the most economically important berry crops worldwide due to its high consumer demand and exceptional nutritional value. Strawberry fruits are rich in vitamin C, anthocyanins, flavonoids, phenolic compounds, and other bioactive metabolites that contribute to human health and enhance their commercial appeal. The expansion of protected cultivation systems and soilless production technologies has further increased strawberry cultivation in many regions, reinforcing its significance within the horticultural sector (Passa et al., 2026).

Successful strawberry production depends on favorable environmental conditions and the availability of high-quality irrigation water. However, increasing water scarcity and climate change have intensified the use of alternative irrigation sources, many of which contain elevated concentrations of dissolved salts. Consequently, salinity has emerged as one of the major environmental constraints affecting sustainable strawberry cultivation, particularly in arid and semi-arid regions (Passa et al., 2026; Georgiadou et al., 2025).

Soil and water salinization represent a growing challenge for global agriculture. Salinity develops through the accumulation of soluble salts in the root zone, often resulting from poor-quality irrigation water, inadequate drainage, intensive fertilization practices, and climate-related processes. Elevated salt concentrations reduce water availability to plants and interfere with nutrient acquisition, ultimately limiting plant growth and productivity. As reliance on marginal-quality water resources continues to increase, salinity is expected to become an even more significant limitation to strawberry production in the future (Passa et al., 2026).

Among horticultural crops, strawberry is considered particularly vulnerable to saline conditions. Even relatively low salinity levels can adversely affect plant performance due to the crop's shallow root system and limited capacity to maintain ionic balance. Excessive accumulation of sodium (Na^+) and chloride (Cl^-) ions disrupts nutrient uptake and transport by competing with essential elements such as potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}). This ionic imbalance impairs enzyme activity, membrane stability, and several metabolic processes necessary for normal plant development (Passa et al., 2026).

In addition to ionic disturbances, salinity reduces the soil water potential, restricting water absorption by roots and inducing physiological drought. Reduced water availability limits cell expansion and leaf development, while prolonged exposure to saline conditions promotes the generation of reactive oxygen species (ROS), resulting in oxidative damage to cellular structures and metabolic functions (Ali et al., 2025; Passa et al., 2026; Georgiadou et al., 2025).

The combined effects of ionic toxicity, osmotic stress, nutrient imbalance, and oxidative damage significantly suppress vegetative growth in strawberry plants. Salinity has been associated with reductions in leaf area, crown diameter, root development, and total biomass accumulation. Furthermore, symptoms such as leaf chlorosis, marginal necrosis, and premature senescence are frequently observed under severe saline conditions, contributing to reduced plant vigor and establishment (Passa et al., 2026).

At the physiological level, salinity negatively affects photosynthetic performance and plant water relations. Osmotically induced stomatal closure restricts carbon dioxide assimilation, while excessive ion accumulation damages chloroplast structure and disrupts photosynthetic electron transport. Salinity also alters chlorophyll content, transpiration rates, nutrient metabolism, and water-use efficiency. To survive under stress conditions, plants divert substantial metabolic resources toward protective mechanisms, including osmotic adjustment and antioxidant defense, often at the expense of growth and reproductive development (Georgiadou et al., 2025; Passa et al., 2026).

These physiological disturbances ultimately lead to substantial reductions in yield and fruit quality. Salinity commonly decreases flower production, fruit set, fruit size, and total marketable yield. Reduced biomass accumulation limits carbohydrate availability for fruit development, resulting in fewer and smaller fruits. Excessive salt accumulation may also negatively affect fruit appearance, firmness, and commercial value. Although moderate salinity has occasionally been associated with increases in soluble solids content and certain antioxidant compounds, these benefits rarely compensate for the overall decline in productivity observed under prolonged saline conditions (Lu et al., 2025; Passa et al., 2026).

Given the increasing severity of salinization and the economic importance of strawberry cultivation, the development of sustainable strategies to mitigate salinity-induced damage has become a major research priority. Current approaches include the development of salt-tolerant cultivars, the application of beneficial microorganisms and biostimulants, grafting technologies, and improved irrigation and nutrient management practices. A thorough understanding of the physiological and molecular responses of strawberry plants to salinity is therefore essential for ensuring sustainable production under increasingly challenging environmental conditions (Passa et al., 2026; Georgiadou et al., 2025).

1.2 Oxidative and osmotic stress under salinity

Among the various physiological disorders induced by salinity, osmotic stress and oxidative stress are considered the principal mechanisms responsible for growth inhibition and metabolic dysfunction in strawberry plants. These interconnected processes disrupt cellular homeostasis, impair physiological performance, and reduce the ability of plants to adapt to saline environments (Passa et al., 2026).

Osmotic stress represents one of the earliest responses to salt exposure. The accumulation of soluble salts in the rhizosphere lowers soil water potential, making water uptake by roots increasingly difficult despite the presence of adequate soil moisture. As a result, plants experience physiological drought, characterized by reduced cell turgor, stomatal closure, diminished photosynthetic activity, and restricted growth. To maintain water balance and cellular function under these conditions, plants activate osmotic adjustment mechanisms involving the accumulation of compatible solutes such as proline, soluble sugars, and other osmoprotective compounds (Passa et al., 2026).

Prolonged osmotic stress, together with salinity-induced ionic imbalance, stimulates the excessive production of reactive oxygen species (ROS). Although ROS are continuously generated during normal metabolic processes and function as signaling molecules at low concentrations, their accumulation under stress conditions can exceed the detoxification capacity of antioxidant defense systems. This overproduction results in oxidative stress, causing damage to proteins, nucleic acids, photosynthetic pigments, and membrane lipids, thereby compromising cellular integrity and physiological performance (Hasanuzzaman et al., 2021).

Major ROS generated under saline conditions include superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\bullet OH$), and singlet oxygen ($^1O_2$). Among these molecules, H_2O_2 plays a dual role in plant stress responses. At controlled concentrations, it functions as a signaling molecule involved in the regulation of stress-responsive genes and activation of antioxidant defense pathways. However, excessive H_2O_2 accumulation contributes directly to oxidative injury, membrane deterioration, and cellular dysfunction. Studies in strawberry have demonstrated that elevated H_2O_2 levels are closely associated with reduced physiological performance under salinity, whereas enhanced antioxidant activity can alleviate oxidative damage and improve stress tolerance (Passa et al., 2026; Hasanuzzaman et al., 2021).

One of the most reliable indicators of oxidative stress is lipid peroxidation, a process in which ROS attack polyunsaturated fatty acids within cellular membranes. This process results in reduced membrane fluidity, increased permeability, and loss of cellular compartmentalization. Malondialdehyde (MDA), a major end-product of lipid peroxidation, is widely used as a biochemical marker of oxidative damage. Increased MDA concentrations have consistently been reported in salt-stressed strawberry plants, reflecting severe membrane injury and impaired cellular integrity (Hasanuzzaman et al., 2021; Passa et al., 2026).

To counteract the detrimental effects of osmotic and oxidative stress, strawberry plants activate several protective mechanisms. Among these, proline accumulation is considered particularly important. Beyond its role in osmotic adjustment, proline contributes to the stabilization of proteins and cellular membranes, scavenges reactive oxygen species, and supports cellular redox balance. Enhanced proline accumulation has been frequently observed in salt-stressed strawberry cultivars and is commonly associated with improved tolerance to saline conditions (Passa et al., 2026).

Recent studies have also highlighted the regulatory role of nitric oxide (NO) in salinity adaptation. Nitric oxide functions as a multifunctional signaling molecule involved in antioxidant defense, ion

homeostasis, osmotic regulation, and stress-responsive gene expression. Evidence suggests that NO interacts closely with H₂O₂, forming an integrated signaling network that coordinates downstream defense responses. Yang et al. (2025) demonstrated that improved salt tolerance in strawberry was associated with a NO–H₂O₂ signaling circuit regulated by the transcription factors FaWRKY70 and FaWRKY40. Activation of this pathway enhanced antioxidant capacity reduced oxidative damage, and improved physiological adaptation under saline conditions (Yang et al., 2025).

Overall, salinity-induced osmotic and oxidative stress represent central components of the plant response to saline environments. Reduced water availability, ionic imbalance, and excessive ROS generation collectively impair cellular function and plant performance. In response, strawberry plants activate adaptive mechanisms including osmotic adjustment, antioxidant defenses, proline accumulation, and NO-mediated signaling pathways to preserve cellular homeostasis and enhance stress tolerance (Passa et al., 2026; Yang et al., 2025).

1.3 Plant priming as a mitigation strategy

Plant priming is a physiological phenomenon through which plants are exposed to specific stimuli that prepare them to respond more rapidly and effectively to subsequent biotic or abiotic stresses. Rather than continuously activating defense mechanisms, primed plants maintain a heightened state of alertness, enabling faster and stronger protective responses when stress occurs (Conrath et al., 2006; Hilker et al., 2016). This process involves molecular, biochemical, and physiological adjustments, including changes in antioxidant metabolism, phytohormone signaling, gene expression, and epigenetic regulation, which collectively enhance plant adaptability and stress tolerance (Kerchev et al., 2020; Jatana et al., 2024). The beneficial effects of priming are closely associated with the establishment of stress memory, allowing plants to respond more efficiently to future stress events through enhanced antioxidant activity, improved osmotic adjustment, better photosynthetic performance, and more effective ROS detoxification systems (Bruce et al., 2007; Hilker et al., 2016; Kerchev et al., 2020; Wang et al., 2022). In some cases, stress memory may persist throughout plant development and even be transmitted across generations through epigenetic mechanisms (Molinier et al., 2006; Wang et al., 2022).

Plant priming has attracted considerable attention as a sustainable agricultural strategy because it enhances crop resilience while reducing dependence on agrochemical inputs (Jatana et al., 2024; Sahoo et al., 2025). Priming treatments are generally applied at low doses as seed,

foliar, or root-zone treatments and contribute to improved resource-use efficiency and yield stability under adverse environmental conditions (Liu et al., 2022). Consequently, priming is increasingly recognized as an important tool for climate-smart agriculture, particularly in addressing challenges associated with drought, salinity, heat stress, and other environmental constraints (Balmer et al., 2015; Sahoo et al., 2025). Among the various priming agents investigated, salicylic acid (SA) is one of the most extensively studied due to its central role in plant defense signaling and stress adaptation. SA has been shown to enhance antioxidant activity, improve photosynthetic performance, and increase tolerance to abiotic stresses, including salinity and drought (Kerchev et al., 2020). In recent years, biopolymer-based materials have also emerged as promising tools for plant stress management. Sodium alginate, a biodegradable and biocompatible polysaccharide, has demonstrated biostimulant properties and has been associated with improved physiological responses and stress resilience in strawberry cultivation (Georgiadou et al., 2025). Furthermore, advances in nanotechnology have enabled the development of novel delivery systems based on natural polymers such as zein, which can improve the stability and controlled release of bioactive compounds. The incorporation of graphene oxide (GO) into polymer matrices has also attracted attention due to its ability to enhance the physicochemical properties and functionality of agricultural formulations.

Strawberry (*Fragaria × ananassa* Duch.) is particularly sensitive to environmental stresses such as salinity, drought, high temperatures, and water scarcity, which negatively affect plant growth, photosynthesis, fruit quality, and productivity. Recent studies have demonstrated that priming treatments can improve strawberry tolerance to abiotic stress by enhancing antioxidant defenses, water-use efficiency, and physiological performance (Georgiadou et al., 2025). Therefore, the development of innovative and sustainable priming strategies represents an important approach for improving strawberry cultivation under increasingly challenging environmental conditions.

The present study followed an experimental design consisting of 23 distinct treatments aimed at evaluating the effectiveness of novel encapsulated formulations of salicylic acid (SA), as well as hybrid systems based on zein nanoparticles and functionalized sodium alginate (NaA) with graphene oxide (GO), under salinity stress conditions. Therefore, the aim of the present study was to evaluate the efficacy of encapsulated and functionalized biopolymer-based formulations as sustainable tools for improving salt stress tolerance in strawberry plants. Specifically, the study investigated the ability of zein-encapsulated salicylic acid (Zein-SA) and sodium alginate–graphene oxide (functionalized NaA–GO) formulations to alleviate salinity-induced oxidative and osmotic stress, improve physiological performance and plant growth, and enhance overall

resilience of strawberry plants under saline conditions. By assessing these innovative delivery systems, the study seeks to contribute to the development of environmentally friendly strategies for sustainable strawberry production in salt-affected agricultural environments.

1.4 Salicylic acid and zein encapsulation

Salicylic acid (SA) is a naturally occurring phenolic phytohormone that plays a central role in regulating plant growth, development, and responses to both biotic and abiotic stresses. It functions as a signaling molecule involved in the activation of defense pathways, antioxidant metabolism, and stress-responsive gene expression. Under adverse environmental conditions, such as salinity, drought, temperature extremes, and pathogen attack, endogenous SA levels increase, triggering physiological and biochemical mechanisms that enhance plant resilience (Xin et al., 2025; Tian et al., 2025). To overcome these limitations, nanotechnology-based delivery systems have been proposed. Among these, zein nanoparticles have attracted considerable attention. Zein is a biodegradable, biocompatible, and Generally Recognized as Safe (GRAS) storage protein derived from maize (*Zea mays*). Due to its hydrophobic nature and excellent film-forming properties, zein can self-assemble into nanoscale particles capable of encapsulating bioactive compounds, including plant growth regulators such as salicylic acid. These nanoparticles protect the encapsulated molecules from environmental degradation while improving their physicochemical stability (Polyakov et al., 2023). Encapsulation of SA within zein nanoparticles can substantially improve its effectiveness through several mechanisms. First, the zein matrix protects SA against photodegradation, oxidation, and premature loss after application. Second, nanoparticle-based formulations facilitate better adhesion to plant surfaces and enhance penetration through the cuticle, resulting in improved uptake efficiency. Third, zein nanoparticles provide a controlled and sustained release of SA, allowing the hormone to be delivered gradually over an extended period rather than being released immediately after application. This prolonged availability can maintain plant defense responses for longer durations and reduce the need for repeated applications (Polyakov et al., 2023; Zhang et al., 2023). The controlled release behavior of zein-encapsulated SA is particularly relevant for strawberry cultivation, where plants are frequently exposed to environmental stresses such as salinity, drought, and temperature fluctuations. A sustained supply of SA may continuously stimulate antioxidant defenses, osmotic adjustment mechanisms, and stress-responsive signaling pathways, thereby improving overall plant performance under stress conditions. Furthermore, nanoencapsulation can reduce the total amount of SA required to achieve the desired