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Review

Plant-Derived Biostimulants and Liposomal Formulations in Sustainable Crop Protection and Stress Tolerance

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Abstract

Plant-derived biostimulants represent an innovative approach to enhancing crop productivity, resilience, and quality within sustainable agricultural systems by improving nutrient uptake, stress tolerance, and plant defense mechanisms while reducing reliance on synthetic inputs. However, their effectiveness is often limited by poor stability and low bioavailability. Recent advances in nanotechnology, particularly liposomal formulations, address these limitations by enhancing the stability, solubility, and delivery efficiency of bioactive plant compounds. Liposomes facilitate the penetration and systemic transport of active ingredients within plant tissues and enable controlled release at the target site, thereby increasing biostimulant efficacy. This review summarizes current knowledge on plant-derived biostimulants, their classification, nano-formulation, molecular mechanisms, and roles in mitigating abiotic and biotic stress. Special emphasis is placed on liposome-based formulations, including supercritical CO_2 extracts and nano-liposomal delivery systems, with examples such as garlic extract and the EliceVakcina® complex. Finally, the potential of liposomal technologies in integrated crop protection and sustainable agriculture is discussed.

Keywords: biostimulants; plant extracts; nano-liposomes; nano-biopesticides; abiotic stress; biotic stress; sustainable agriculture

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1. Introduction

The growing global demand for safe and high-quality food has intensified the need for sustainable agricultural strategies. Conventional crop protection systems largely depend on synthetic agrochemicals, which raise increasing concerns related to environmental contamination, human health risks, and the development of pesticide resistance. These challenges have accelerated interest in plant-derived biostimulants and biopesticides as environmentally friendly alternatives capable of enhancing crop resilience, stress tolerance, and productivity. However, the practical effectiveness of many plant-based products is often constrained by limited stability, poor solubility, and inefficient delivery of bioactive compounds. In this context, nanotechnology—particularly liposome-based formulations—offers innovative solutions by improving the stability, bioavailability, and targeted delivery

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of plant-derived bioactives. Owing to their nanoscale size and biodegradable lipid composition, liposomal formulations enable effective plant uptake and systemic transport of active ingredients at lower application doses. This review summarizes current advances in plant biostimulants (PBs) and nanotechnology-assisted delivery systems, with a particular focus on the liposomal encapsulation of bioactive plant extracts for sustainable crop protection.

Given the rapidly growing body of literature on plant biostimulants, several recent reviews have comprehensively summarized their roles in enhancing abiotic stress tolerance, elucidated underlying mechanisms of action, and discussed formulation strategies, including polymer-based and microencapsulation approaches. These studies highlight the increasing interest in improving the efficacy and stability of biostimulant products under diverse environmental conditions. Recent reviews detail how plant-derived biostimulants enhance crop resilience to abiotic stresses such as drought, salinity, and temperature extremes through modulation of hormonal signaling, antioxidant systems, and stress-responsive gene networks. In parallel, formulation-focused studies demonstrate that encapsulation strategies—including alginate microencapsulation and biopolymer or nano-based matrices—significantly improve the stability, controlled release, and agronomic effectiveness of liquid biostimulants under practical application conditions [1–4]. In addition, the present review specifically focuses on liposome-based nanoformulations of plant-derived biostimulants, emphasizing their unique delivery properties, systemic translocation, and potential applications in sustainable crop protection.

The literature included in this review was identified through targeted searches in Web of Science, Scopus, and Google Scholar using combinations of keywords such as ‘plant biostimulants’, ‘liposomal formulation’, ‘encapsulation’, ‘nano-biostimulants’, and ‘plant stress tolerance’. Studies published between 2019 and 2024 were prioritized.

2. Organic Agriculture and Regulatory Frameworks

2.1. The Significance of Organic Agriculture Globally and in the European Union

In the European Union, organic farming is governed by a harmonized regulatory system that restricts the use of synthetic pesticides and fertilizers, thereby encouraging the application of natural products such as plant-derived biostimulants. These products are recognized for their capacity to enhance nutrient use efficiency, stress tolerance, and crop quality without direct pesticidal activity. The EU regulatory framework for fertilizing products and biostimulants also influences the development of innovative formulations, including nano-enabled and liposome-based systems. Although nano-specific regulations are still evolving, current legislation requires appropriate documentation of formulation characteristics relevant to safety and performance [5,6].

The proportion of organic farmland is increasing on every continent. According to the most recent global data collected from 190 countries (2023), more than 99 million hectares of farmland are under organic management (Figure 1). Globally, the area of land under organic cultivation has increased sevenfold over the past 20 years (“The World of Organic Agriculture 2025”, published by the Research Institute of Organic Agriculture FiBL, Brussels, Belgium and IFOAM—Organics International, Nuremberg, Germany https://www.fibl.org/en/shop-en/1797-organic-world-2025 (accessed on 3 August 2025)).

Globally, the regions with the largest organic farming area are Oceania (53.2 million hectares), Europe (19.5 million), Latin America (10.3 million), Asia (9.1 million), Africa (3.4 million) and North America (3.3 million). The second-highest organic shares of the total agricultural land were in Europe (3.9%; European Union: 10.9%) after Oceania (14.1%). Although the top three countries by organic area were Spain (2.99 million hectares), France (2.77 million hectares) and Italy (2.46 million hectares), 16 countries had more than 10% of their farmland under organic management in Europe (Research Institute of Organic Agriculture FiBL, Brussels, Belgium and IFOAM—Organics International, Nuremberg, Germany).

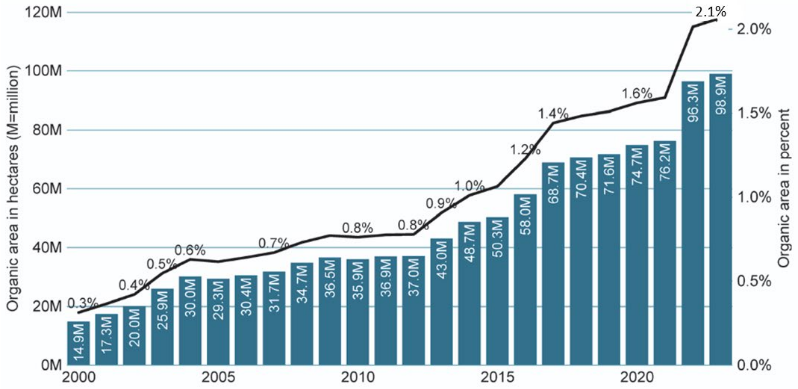


Figure 1. World: Growth of the organic agricultural land and organic share 2000–2023 (source: FiBL-IFOAM surveys 2001–2023).

2.2. Types of Fertilizing Products, Plant Conditioners, and Plant Protection Preparations, and the Regulation of Their Use in Organic Farming

Fertilizing products have been experimentally proven to exert beneficial effects on the soil and cultivated plants, without causing toxic impacts on the environment or the crop itself. The EU Regulation 2019/1009 harmonized previous legislation, thereby creating a unified market for fertilizing products within the European Union. It introduced uniform safety, quality, and labelling requirements, and also set toxic contaminant limits for fertilizing products. This new EU regulation established functional product categories such as fertilizers (organic, inorganic), liming materials, soil improvers (organic, inorganic), growing media, inhibitors (of nitrification, denitrification, and urease), PBs, and fertilizing product blends (https://eur-lex.europa.eu/eli/reg/2019/1009/oj/eng (accessed on 20 November 2024)).

The concept of PB was introduced in Regulation (EC) No 1107/2009, describing products that stimulate plant nutrition processes independently of their nutrient content, with the aim of improving one or more characteristics of the plant or its rhizosphere (https://eur-lex.europa.eu/eli/reg/2009/1107/oj/eng (accessed on 21 November 2022)). PBs enhance nutrient use efficiency, abiotic stress tolerance, crop quality, and the availability of assimilable nutrients. In organic farming, the use of PBs for plant protection purposes is regulated under Regulation (EC) No 1107/2009. The regulation approved the use of certain plant-based raw materials, extraction agents, and plant-active compounds that were not originally developed for plant protection purposes, provided they are useful for reducing damage and are not toxic to the crop [7].

2.3. Regulatory Context of Liposomal Nano-Formulations in the European Union

Under the current European Union legal framework, liposomal nano-formulations may fall under two major regulatory regimes depending on their primary function: plant protection products or plant biostimulants. If the formulation is intended to act as a pesticide or disease-control agent, it is regulated under Regulation (EC) No 1107/2009 on the placing of plant protection products on the market (EUR-Lex). In this case, the nanoscale characteristics of the formulation must be explicitly assessed. The European Food Safety Authority (EFSA) has published detailed guidance on the risk assessment of nanomaterials in regulated products, which sets requirements for particle characterisation, exposure assessment, and environmental fate (EFSA 2021 Guidance, https://www.efsa.europa.eu/en/efsajournal/pub/6768 (accessed on 3 August 2021)). Liposomal carriers, being nanostructured lipid systems, are, therefore, subject to nanospecific data requirements during authorisation and risk evaluation.

When the same formulation is intended to enhance plant growth, nutrient uptake, or stress tolerance without exerting a direct protective effect, it can be classified as a plant biostimulant under Regulation (EU) 2019/1009, the Fertilising Products Regulation (FPR) (EUR-Lex). The FPR defines "plant biostimulant" (Product Function Category 6) and establishes harmonised criteria for CE-marking and market access. Although the regulation does not yet contain explicit nano-specific provisions, operators are required to document any nano-features of the formulation (e.g., particle size distribution, stability, release profile) in the technical dossier, in accordance with the Commission's 2022 Recommendation on the definition of nanomaterial [8].

Several recent policy analyses have highlighted a regulatory gap for nano-enabled agrochemicals: while nano-specific safety assessment is mandated under the plant protection products framework, it remains less defined under the FPR for biostimulants [9]. Consequently, liposomal nano-biostimulants occupy an intermediate regulatory position—scientifically characterised as nanomaterials, but governed by evolving policy instruments rather than a dedicated nano-specific legal framework. Continued harmonisation of EFSA guidance and the FPR is, therefore, essential to ensure consistent risk assessment and transparent market authorisation of liposomal nano-formulations in sustainable agriculture.

2.4. Biopesticides

Although EU regulations do not address the concept and subject of biofertilizers and biopesticides, they are central topics in research. Chemical plant protection, which has been widespread since the 1960s, has greatly contributed to increasing agricultural productivity, although the procedures have had harmful effects, such as resistance to pesticides and the presence of pesticide residues in crops and the environment. Therefore, it has become important to develop alternatives to chemical plant protection. It is important to achieve the highest possible crop yields while protecting crops and the environment, given the declining natural resources. Biofertilizers and biopesticides are preparations made from natural materials that control pests in an environmentally friendly way, so their use can play a major role in the development of sustainable agriculture [10]. In terms of their basic ingredients, biopesticides can be microbial biopesticides, transgenic plants containing special protective substances, and biochemical biopesticides (essential oils, plant growth regulators, insect growth inhibitors, secondary metabolites, and natural minerals) [11–13].

Nowadays, plant-based agents belonging to the group of biopesticides are playing an increasingly important role [14]. It is known that the essential oils of thyme, mint, oregano and other medicinal plants have a strong germination-inhibiting effect as bioherbicides [15,16]. Plant-based biofungicides are produced from the essential oils of algae, lichens, and angiosperm species, which suppress the growth of plant pathogenic fungi and disrupt their gene regulation [17]. Supercritical carbon dioxide (SC-CO_2) extracts of clove (*Syzygium aromaticum* L.) and cinnamon (*Cinnamomum cassia* L.), containing eugenol, cinnamaldehyde, etc., have been successfully used as biofungicides in the treatment of strawberries infected with gray mold (*Botrytis cinerea*) in an in vitro leaf disc test [18]. The use of various plant extracts (lavender, fennel, eucalyptus, etc.) has been shown to have a repellent effect, cause molting disorders, and cell toxicity in insect pests [19–21]. Studies describing the effect of plant extracts on agricultural pests also mention their disadvantages, as their poor physical and chemical stability, high volatility, and thermal decomposition make them irregular and slow in their effectiveness [22].

2.5. Categories of Plant Biostimulators and Their Use as Plant Conditioners

Du Jardin (2015) classified PBs into seven categories: humic and fulvic acid, protein hydrolysates and other N-containing compounds, seaweed extracts and botanicals, chitosan and other polymers, inorganic compounds, beneficial fungi and beneficial bacteria. Plant-based conditioners belonging to the group of PBs potentially offer new approaches for regulating physiological and molecular processes, stimulating growth, enhancing nutrient uptake, increasing crop yield, and alleviating stress-induced limitations [23–25].

In another definition, biostimulants are formulated products of biological origin that enhance plant productivity due to the emerging or synergistic properties of their complex components. Thus, biological functions can be positively modulated by mixtures of molecules that often do not have a clear site of action, and in many cases, their mechanism of action is not yet fully understood, although efforts should be made to identify at least their modes of action [26].

3. Plant-Derived Biostimulators

Plant-based biostimulants can be divided into two major groups: seaweed extracts [27] and extracts derived from various plant parts, which are widely used to improve crop production. Extracts from different plant organs (roots, shoots, seeds) elicit distinct responses depending on the mode of application, concentration, and plant species. These extracts originate from representatives of various plant families such as *Amaryllidaceae*, *Brassicaceae*, *Ericaceae*, *Fabaceae*, *Fagaceae*, *Moringaceae*, *Plantaginaceae*, *Poaceae*, *Rosaceae*, *Solanaceae*, *Theaceae*, and *Vitaceae* [26]. There is little information on the composition of the developed biostimulator complexes; patents often hide them, but researchers have investigated their effects. Using a growth regulator of plant origin, a complex of biologically active compounds (organic acids, phytohormones, and oligosaccharides) elevated the levels of auxin and cytokinin phytohormones, but reduced the abscisic acid (ABA) content in wheat. Moreover, this biostimulator reduced the infection by *Phytophthora infestans*, *Erwinia carotovora* and *Rhizoctonia* sp. in potato tubers during storage [28,29]. A protein hydrolysate was produced from sunflower defatted seed meal, which helps the plant root elongation of *Lepidium sativum* and *Lactuca sativa* seedlings [30].

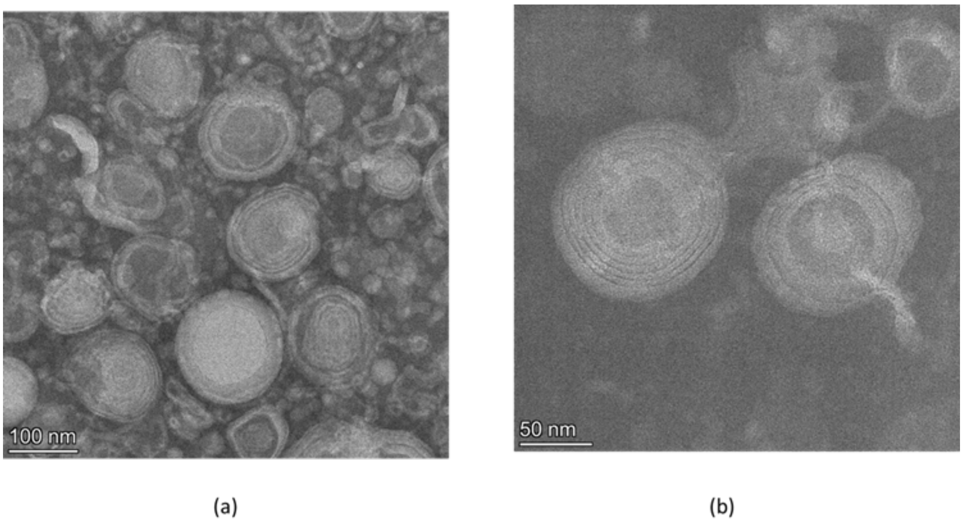
3.1. Bioactive Compounds of Plant-Biostimulators

Different plant parts have distinct chemical compositions, and when applied externally, they influence plant growth and stress tolerance. They may contain phytohormones, nutrients, as well as antifungal and antimicrobial compounds that protect plants against biotic and abiotic stress. Plants are rich sources of bioactive compounds and secondary metabolites, including nitriles, flavonoids, alkaloids, and antioxidants. Plant extracts comprise both organic and inorganic compounds, such as mineral elements (potassium, calcium, sulfur, magnesium, phosphorus), vitamins, amino acids, and diverse phytohormones [26,31,32].

In the following studies, biostimulant effects were typically evaluated using controlled greenhouse or field experiments, where treated plants were compared to untreated or water-treated controls. Physiological parameters, yield traits, stress-related metabolites, and gene expression profiles were commonly assessed to determine treatment efficacy.

3.2. An Example for a Complex of Plant-Extracts: EliceVakcina Biostimulator

EliceVakcina (former name ELICE16INDURES) is a complex biostimulant containing 11 medicinal plant extracts formulated in liposomal carriers [33] (Figure 2). Of the 11 herbs, ginger, thyme, and garlic were detected as biostimulants.



(a) (b)

Figure 2. Transmission electron microscope (TEM) image of the structure of EliceVakcina, liposomes, (**a**) bar = 100 nm, (**b**) bar = 50 nm. TEM recordings showed a multilamellar liposome structure in the range of 100–200 nm [33].

While the extracts are well known in human medicine, their application as PBs was a new development in 2019 by the Research Institute for Medicinal Plants and Herbs, Hungary. Studies on its herbal components demonstrated their capacity to improve growth, yield, antioxidant activity, and drought tolerance in several crop species, suggesting that EliceVakcina may have similar potential [33,34]. For instance, ginger extract (*Zingiber officinale* Roscoe), when applied as a biostimulant, positively influenced the developmental processes of *Rosa damascena* Mill. plants by increasing flower number, flower yield, relative water content, and total chlorophyll content, as well as the essential oil content and yield of the roses [35]. In another study, strawberry (*Fragaria* × *ananassa* Duch.) shoots treated with aqueous thyme extract (*Thymus capitatus*) during the in vitro rooting phase exhibited significant increases in both shoot and root development. In addition, phenolic content, peroxidase (POD) activity, and antioxidant activity were enhanced [36].

Seed coating with biostimulants is considered a promising approach in crop production to enhance tolerance to biotic and abiotic stresses. For example, durum wheat (*Triticum turgidum* L. subsp. *durum* (*Desf*) *Husn.*) seeds coated with aqueous thyme extract (*Thymus capitatus*) showed improved germination rate, seedling growth, and activation of plant signaling pathways through the induction of the ABA pathway. Furthermore, in pot experiments, irrigation with thyme extract promoted root and shoot development, increased nitrogen balance index, chlorophyll, ABA, anthocyanin, and flavonoid content in leaves. During the later growth stage, plants subjected to drought stress demonstrated improved defense strategies when irrigated with thyme extract, including enhanced root elongation, reduced dry matter and chlorophyll loss in shoots and roots, and maintenance of balanced nitrogen metabolism [37].

The main herbal component of EliceVakcina is the garlic extract. Garlic (*Allium sativum* L.) is a well-known medicinal plant that contains numerous beneficial bioactive compounds, including polysaccharides, organic sulfides, phenolic compounds, and saponins [38–40].

Despite these promising results, the large-scale application of liposome-formulated biostimulants such as EliceVakcina may face practical challenges, including production scalability, formulation costs, and the need for precise timing and dose optimization under field conditions. Further field-level studies and techno-economic evaluations are, therefore, required to support broader agricultural adoption.

3.3. Garlic-Extract as Biostimulator

There are numerous studies on treatments with various plant-based biostimulants, but there is little literature available on the use of garlic extract as a biostimulant. The antimicrobial effect of garlic has been reported in in vitro experiments, where it was effective against a number of plant pathogenic fungi and bacteria [41–44]. Allicin is produced from the amino acid alliin (S-allyl cysteine sulfoxide) during tissue damage in a reaction catalyzed by the enzyme alliinase. Allicin can inhibit the growth of both bacteria and fungi in a dose-dependent manner [45,46]. Allicin exhibits rapid membrane permeability and reacts with the free thiol groups of proteins in a thiol-disulfide reaction [47]. The effect regulated by allicin was demonstrated in potato tubers and tomatoes infected with *Phytophthora infestans*, carrots infected with *Alternaria brassisicola*, *Arabidopsis thaliana* infected with *Hyaloperonospora parasitica*, and rice infected with *Magnaporthe grisea* [48]. In addition, allicin has been shown to inhibit the plant pathogens *Agrobacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas* bacteria, as well as *Botrytis*, *Plectosphaerella*, and *Phytophthora* fungi [49].

In a field experiment, the application of aqueous garlic extract (AGE) improved the growth and physiological condition of common beans (*Phaseolus vulgaris* L.) and field beans (*Vicia faba* L.). In addition, an increase in phenols, carbohydrate components, free amino acids, proline content, and endogenous phytohormones, especially auxins, gibberellins, and salicylates, was observed in the treated plants [50,51].

Hayat et al., 2016 [52] examined the aqueous extract of 28 different garlic varieties with varying allicin content on a nutrient medium against the phytopathogenic fungi *Fusarium oxysporum*, *Botrytis cinerea*, *Verticillium dahliae*, and *Phytophthora capsici* determined the minimum inhibitory concentration (MIC) that completely inhibited fungal growth in a microdilution assay (MIC_{50} 1% extract, containing 39 $\mu g\ mL^{-1}$ allicin; MIC_{90} 10%). The eggplant and pepper leaf disc test against *P. capsici* and *V. dahliae* showed a significant reduction in infection at a dose of 100 $mg\ mL^{-1}$ (allicin content 3.9 $mg\ g^{-1}$), with disease severity decreasing from 85% to 12–14%. In addition, the bioactivity of AGE was studied by spraying it on the leaves of cucumber seedlings (*Cucumis sativus*), which altered the plant's defense mechanism by increasing the activity of catalase (CAT), superoxide dismutase (SOD), and POD.

Furthermore, in a greenhouse experiment, AGE leaf spraying of tomato seedlings (*Solanum lycopersicum*) significantly increased plant height, leaf area, stem diameter, and fresh/dry weight. After treatment, the increased metabolism and defensive enzyme induction of tomato plants suggest a 'priming' phenomenon. The overproduction of SOD and CAT induces stress tolerance, and the overexpression of SOD genes results in growth under stress, thus activating the plant's induced defense capacity. At low concentrations, AGE activated antioxidant enzymes, but at higher concentrations, it resulted in increased malondialdehyde accumulation, causing lipid peroxidation and membrane damage [53].

The use of AGE as a biostimulant improved the quality of pepper and eggplant crops, altered the physiological potential of the treated plants, and triggered their defensive responses against *Phytopthora capsici* pathogenic fungal infections. In addition, there was a significant increase in the metabolite content of the treated plants, such as chlorophyll, carotenoids, and soluble sugars. The application of AGE also had a primary effect on pepper plants, inducing defense responses before inoculation with *P. capsici.* Thus, the treated plants successfully resisted infection with the help of their activated antioxidant system, carotenoids, and other protective metabolites. The stress-induced hydrogen peroxide content was extremely low in the treated plants, indicating successful resistance to pathogenic infections [54].

3.4. Plant Biostimulators in Stress Tolerance

Plant extracts, as plant-based biostimulants, are used as pesticides in agriculture, causing morphological, physiological, biochemical, proteomic, and transcriptomic changes in the treated plant. Plant-based biostimulants improve the metabolic and adaptive performance of treated plants under abiotic and biotic stress conditions [55]. Various environmental constraints, abiotic and biotic stresses, limit the yield and quality of crops, and to counteract these effects, plants adapt through certain biochemical changes to maintain proper growth. These include the accumulation of certain osmolytes and osmoprotectants, including proline, glycine betaine, total free amino acids, soluble protein, and soluble sugar [56,57]. Among the various strategies for mitigating the negative effects of abiotic stress on plants, the use of environmentally friendly biostimulants is one of the most promising [58,59]. Plants try to overcome environmental changes with sophisticated detection mechanisms, signaling and acclimation strategies, such as increased production of antioxidant metabolites (e.g., flavonoids, proline, enzymes), which help regulate reactive oxygen species (ROS) to avoid oxidative stress and changes in phytohormone (e.g., ABA and jasmonic acid) levels [60,61]. While these results demonstrate clear biological activity under laboratory and controlled greenhouse conditions, their translation to open-field environments remains insufficiently documented.

3.5. The Transcriptomic Effect of Plant-Based Biostimulators in Abiotic and Biotic Stress

Numerous studies have been conducted on experiments with plant-based biostimulants, but few transcriptomic analyses have been performed to support the results of the treatments [55] (Figure 3). Most transcriptomic studies investigating plant responses to biostimulant treatments rely on comparative experimental designs, where treated and untreated plants are exposed to defined abiotic or biotic stress conditions, followed by RNA sequencing and differential gene expression analysis.

Desoky et al., 2019 [62] studied the effect of applying licorice (*Glycyrrhiza glabra*) root extract to pea seeds using pea plants under salt stress (150 mM NaCl for two weeks). Salt stress reduces seedling growth and increases oxidative stress, but these effects were mitigated in pretreated seedlings. Treatment with the extract increased the transcription of SOD, CAT, ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), and peroxiredoxin (PrxQ) genes, reducing oxidative stress. These genes are involved in the cellular antioxidant system, which maintains ROS homeostasis to mitigate oxidative damage [63,64].

In another salt stress study (200 mM NaCl for 24 h), rocket (*Diplotaxis tenuifolia* L.) plants were treated with borage (*Borago officinalis* L.) aqueous extract applied to the foliage, and the expression of transcription factors (TF) was studied. Among others, DREB2A, MYB30, NAC019, NAC72, NAC19, NAC69, ZIP63, ABF3, HB12, and HB7 showed positive regulation [65], which are ABA-dependent TFs. Among the TFs, MYB30 is involved in germination and stress response processes and in ABA signaling [66], while members of the NAC family are involved in plant development and stress responses, respond to ABA, and promote the functioning of the antioxidant system [67]. Furthermore, ZIP63 regulates the circadian cycle and kinases (SNF1-related protein kinase -SnRK1) through a low-energy response [68], ABF3, which is a TF induced by ABA and osmotic stress [69], and HB12 and HB7, which are also ABA-dependent TFs, mediate the growth response to water deficiency [70].

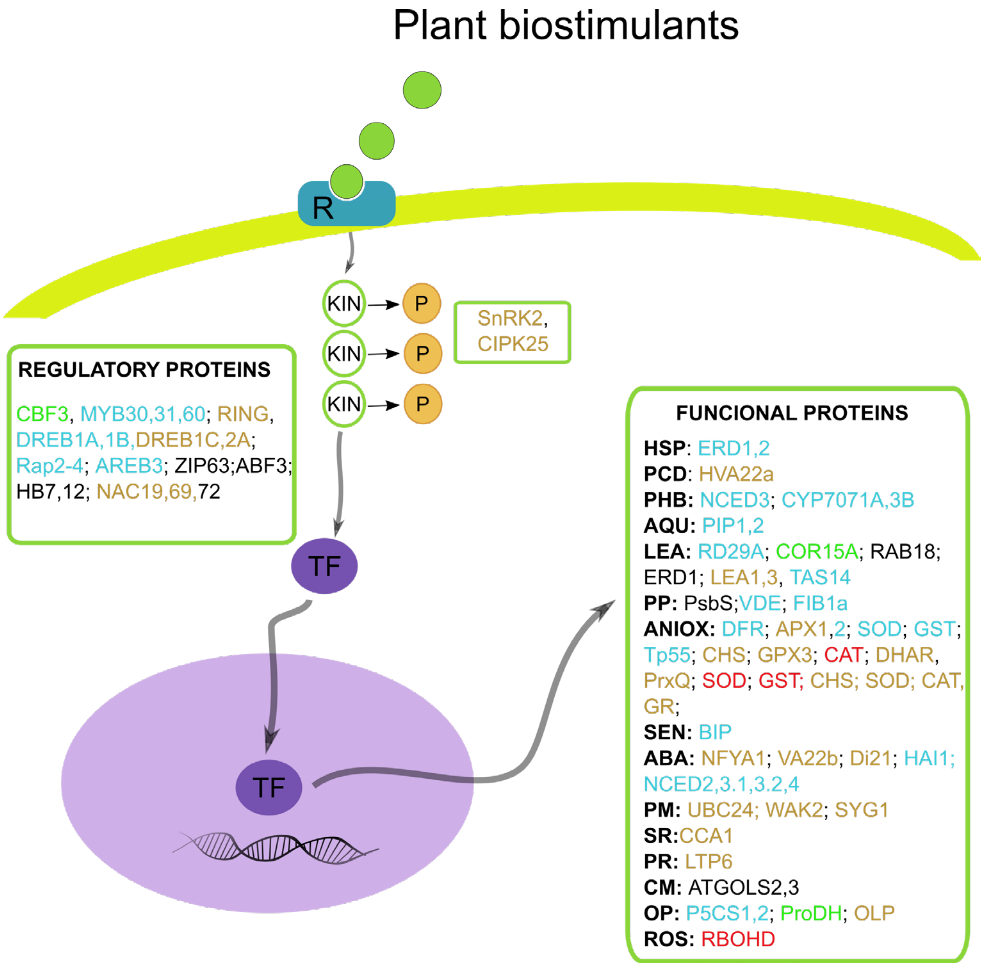


Figure 3. Cellular representation of functional categories of genes differentially expressed in the treated plant when plant-based biostimulants are applied. The listed genes are involved in different types of abiotic stress tolerance, including drought, heat, salt, and cold tolerance. Abbreviations: R: receptor, kin: kinase, P: phosphorus group, TF: transcription factor, HSP: heat shock protein, PCD: programmed cell death, PHB: phytohormone biosynthesis, AQU: aquaporin, LEA: late embryogenesis abundant protein, PP: photoprotection, ANIOX: antioxidant metabolism, SEN: senescence, ABA: ABA biosynthesis and signaling, PM: phenylpropanoid metabolism, SR: stomatal regulation, PR: PR protein, CM: carbohydrate metabolism, OP: osmoprotectant, ROS: reactive oxygen species (source: [55]).

The effect of *Moringa oleifera* ethanol leaf extract was studied on salt-stressed (1000 mg L^{-1}) basil (*Ocimum basilicum*) plants [71], which altered the expression of the osmotin-like protein (OLP) gene and showed a positive correlation with plant growth and yield increase. OLP is a member of pathogenesis-related protein 5 (PR5), which is produced in plants in response to various abiotic and biotic stress factors [72]. Under salt stress and drought conditions, OLP maintains cellular osmolarity through compartmentalization of solutes or structural and metabolic changes [73].

Most studies rely on short-term physiological markers under controlled conditions, which limits direct extrapolation to field-scale agronomic performance. Transcriptomic evidence provides mechanistic support, yet remains available for only a limited number of biostimulant formulations.

4. Nanotechnology in Biostimulator- and Biopesticide Formulation

4.1. Nano-Biopesticides

The formulation of biopesticides/biostimulants is key to their widespread use in agriculture, and nanotechnology is currently one of the most promising technologies in this field. Nano-scale formulation is a widely researched technology in medicine to increase the delivery of active ingredients to target tissues, but it is also used in agronomy today [74–76]. Nanotechnology processes help improve the stability of biopesticides, their delivery to plants, and their transport to the target site within the plant organism [77]. Nano-biopesticides approved for agricultural use are commercially available in many countries, including plant extracts encapsulated in nanostructures (e.g., eugenol, genariol, thymol, etc.), which are nanoemulsions, polymer- or yeast cell-based preparations [22].

Nano-biopesticides differ from conventional biopesticides in size and structure. They are at least 1–100 nm in size [78]. They are particles with active ingredients or designed structures that suppress, mitigate, or prevent damage caused by pests [79]. Nanoparticles (NPs) can be produced by physical (lithography, pyrolysis, milling, mechanical grinding, etc.), chemical (sonochemistry, electrochemistry, microwaves, etc.) and biogenic/'green' synthesis (using living microbes/plants or their extracts/compounds). Widely used nano biomaterials can be classified according to their use as bionanocides (nanocides), nano-sized carrier biopesticides (nanodelivered), biopesticides containing nano-sized components (nanocomposites) or nanodroplet-oriented biopesticides [80].

4.2. Nano-Sized Carrier Biopesticides

Nano-sized carriers are used as carriers for biopesticide active ingredients [81], protecting the active ingredients from premature degradation (photolysis, hydrolysis, biological degradation, etc.) and losses due to leaching and evaporation. This makes them more effective than traditional pesticide formulations [82,83]. Bioactive agents are incorporated into nanocarriers through adsorption, binding to ligands on the outer surface of the nanocarriers, or encapsulation with NPs. During the process, a core-shell arrangement or 'trap' (entrapment) is formed in the pores, layers, matrix or cavities of the nanocarriers. Biopesticide molecules are also delivered encapsulated in nanodrops. In terms of their formulation, the preparations can be nanoemulsions, nanocapsules, nanovesicles, nanogels, nanofibers, etc.

The production of biopesticides using nanocarriers is a very promising solution because the negative physical and chemical properties associated with bioactive ingredients can be managed. These properties include low water solubility, high evaporation rates, and environmental oxidation [80,84]. In addition, the use of this process makes it possible to control the release rate of bioactive substances, e.g., by means of temperature, pH, the ionic strength of the release medium, or biotic and/or abiotic stressors [85–87]. NP carriers used today promote the penetration and effectiveness of pesticide active ingredients, while being non-toxic to the environment [88]. NPs coated with polyethylene glycol (PEG) containing garlic essential oil showed insecticidal activity against the red flour beetle (*Tribolium castaneum*), which was evident five days after treatment and remained above 80% after five months, presumably due to the slow and sustained release of the active components from the NPs. In contrast, the efficacy of free garlic essential oil without encapsulation was only 11% at a similar concentration. This indicates that PEG-coated NPs filled with garlic essential oil can be used to control pests in stored products [89]. *Melissa officinalis* essential oil was used to make a chitosan biopolymer-based nanocomposite film for food packaging, and its antimicrobial effect against *Escherichea coli* was confirmed [90]. Clove (*Syzygium aromaticum*) essential oil nanoemulsion showed a high degree of mycelial growth inhibition (53–82%) against the ascomycete fungus *Neoscytalidium dimidiatum* isolated from *Carum carvi* L. [91].

Organic materials, such as synthetic and natural polymers [92–96], lipids [22,97], plant-derived NPs [98–100], and inorganic materials (silicon dioxide, carbon, calcium, and clay) [86,101]. Encapsulation can be achieved through electrostatic complexation interactions or covalent bonding, which improves uptake, dispersibility, mobility, adhesion, and controlled or targeted release [95].

Several studies have demonstrated the potential of nano-based delivery systems to enhance the bioactivity, stability, and target specificity of plant-derived compounds. Table 1 summarises representative examples of plant extracts formulated as nano-biostimulants or nano-biopesticides, highlighting the main types of nano-carriers used and their associated antifungal, antibacterial, antiviral, and insecticidal effects. Such comparative overviews emphasise the versatility of lipid-based systems, particularly liposomes, in protecting bioactive compounds and improving their efficacy in sustainable crop protection.

Table 1. Summary of representative examples of plant extracts formulated as nano-biostimulants or nano-biopesticides, highlighting the main types of nano-carriers used and their associated antifungal, antibacterial, antiviral, and insecticidal effects.

Plant Extract, Active Compound	Nano-Formulation Type	Main Biological Target Effect	Reference
Garlic (*Allium sativum*) allicin, essential oil	Liposomes; PEG-coated nanoparticles	Antibacterial (*E. coli*, *Listeria*), antifungal (*Penicillium* spp.), insecticidal (*Tribolium castaneum*)	[102–105]
Clove (*Syzygium aromaticum*) eugenol	Nanoemulsion; solid-lipid nanoparticles	Antifungal (*Botrytis*, *Aspergillus*), antioxidant	[91,106–109]
Cinnamon (*Cinnamomum cassia*) cinnamaldehyde	Liposomes; nanoemulsion	Antifungal, antiviral (broad-spectrum)	[110–114]
Ginger (*Zingiber officinale*) gingerol, shogaol	Liponiosomes; nanocapsules	Antifungal (*Aspergillus* spp.), antioxidant enhancement	[115]
Thyme (*Thymus capitatus*) thymol, carvacrol	Nanoemulsion; seed coating (biostimulant)	Drought tolerance; enhanced rooting and phenolic metabolism	[37,116,117]
Quercetin (plant flavonoid)	Lecithin liposomes	Antiviral (Tobacco mosaic virus)	[118]
Ruta graveolens dichloromethane extract	Liposomes; chitosan nanostructure	Insecticidal (*Spodoptera frugiperda*)	[119]
Tagetes erecta, *T. patula* ethanolic extract	Multilamellar liposomes (DPPC)	Insecticidal (*Sitophilus zeamais* larvae)	[120]
Melissa officinalis essential oil	Chitosan ZnO nanocomposite film	Antibacterial (*Escherichia coli*), food-preservative potential	[90]
Moringa oleifera leaf extract	Nanocapsule, foliar nanoformulation	Salinity-stress tolerance; antioxidant enzyme induction	[71,121]

5. Liposomal Formulations

Liposomes are widely used as drug carriers in human medicine, cosmetics and the food industry. Generally, liposomes are spherical vesicles with an inner aqueous space surrounded by a layer, usually a phospholipid (phosphatidylcholine (lecithin) or phosphatidylethanolamine (cephalin) or phosphatidylserine) layer [122]. Liposomes are made from natural, biodegradable lipids that are biologically inactive and non-toxic [123]. The vesicles can be single-layered (unilamellar), multi-layered (multilamellar), or multivesicular liposomes, in which multiple vesicles are found within a unilamellar liposome [124–126]. They are widely used for encapsulating drugs, enzymes, vitamins, and bioactive plant extracts [127–129]. Liposomes are at least 25–2500 nm in size. Classification of liposomes by Kyrychenko and Kovalenko (2025) [130]: (i) Small unilamellar vesicles (SUV) which are 20–100 nm, (ii) Large unilamellar vesicles (LUV) which are 100 nm, (iii) Oligolamellar vesicles (OLV) which are 100–1000 nm, (iV) Giant oligolamellar vesicles (GOV) which are 1000 nm, (v) Multilamellar vesicles (MLV) which are 500 nm.

Types of lipid-based nanocapsules include nanoisosomes, nanoemulsions, nanostructured lipid carriers and solid lipid NPs. Liposomes show a high degree of physicochemical stability during storage, both in terms of the nanocapsules themselves and the active ingredients they contain [81,124]. The essential oil of *Zataria multiflora* was encapsulated into nanoliposomes using thin-film evaporation, ethanol injection, and sonication methods. The thin-layer evaporation method produced multilamellar vesicle liposomes that were more stable during storage than liposomes prepared by other methods [131].

In practical applications, liposome-based formulations have primarily been tested under controlled greenhouse or laboratory conditions using foliar spraying, seed coating, or in vitro bioassays. Typical experimental approaches include comparative treatments with free versus liposome-encapsulated extracts, followed by assessments of plant growth, stress-related physiological parameters, pathogen inhibition, or gene expression responses. Although these studies demonstrate clear advantages of liposomal delivery systems, field-scale applications remain limited and require further validation. In the following section, we summarize the main agricultural application areas of liposome-based formulations, including their roles in enhancing the delivery and translocation of bioactive compounds, as well as their antiviral, antibacterial, antifungal, insecticidal, and stress-related effects in crop protection systems.

5.1. Micro- and Nanoencapsulation Strategies: Methods, Characterization, and Practical Considerations

Encapsulation technologies play a key role in improving the stability, bioavailability, and delivery efficiency of plant-derived biostimulants. While both micro- and nanoencapsulation approaches have been applied in agricultural formulations, the present review primarily focuses on nano-scale delivery systems, particularly liposome-based formulations, due to their superior cellular uptake and potential for systemic translocation in plants. Microencapsulation strategies are briefly discussed here for contextual comparison.

Microencapsulation methods commonly rely on biopolymer-based matrices such as calcium alginate, chitosan, or composite polymer systems, which provide physical protection and controlled release of bioactive compounds. These approaches are widely used for liquid biostimulants and microbial inoculants; however, their relatively large particle size may limit penetration into plant tissues. In contrast, nanoencapsulation strategies—including liposome-based systems—enable the encapsulation of both hydrophilic and lipophilic compounds within nanosized carriers composed of biodegradable lipid bilayers [132–135]. Various production techniques are available for encapsulation, depending on the target particle size and formulation requirements. Microencapsulation is often achieved through extrusion, emulsion–gelation, or electrospray-based techniques, whereas nanoencapsulation typically involves thin-film hydration, ethanol injection, microfluidic approaches, or high-pressure homogenization. Commercial systems such as the Nisco systems (Nisco Engineering Inc., Zurich, Switzerland) represent scalable solutions for microformulation development, although alternative industrial-scale technologies are also increasingly explored [136–139].

The efficiency of encapsulation and particle size distribution are critical parameters for both micro- and nanoformulated biostimulants. Encapsulation efficiency is generally determined by quantifying the proportion of bioactive compounds successfully entrapped within the carrier system, while particle size and polydispersity are commonly assessed using dynamic light scattering (DLS), electron microscopy, or nanoparticle tracking analysis. These parameters strongly influence formulation stability, release kinetics, and biological performance [140–144]. Quality assessment of encapsulated formulations typically includes evaluation of physicochemical stability, homogeneity, release behaviour, and reproducibility during storage and application. For nanoformulations, additional considerations such as colloidal stability and interaction with plant surfaces and tissues are particularly relevant [145]. From an industrial perspective, the large-scale production of encapsulated biostimulants remains a key challenge. Although several commercial biostimulant products utilize encapsulation technologies, detailed information on formulation composition, production costs, and pricing is often proprietary. In the case of nano-encapsulated products, scalability, cost-effectiveness, and regulatory compliance represent critical factors that currently limit widespread adoption. Addressing these challenges will be essential for translating nano-encapsulation concepts—particularly liposome-based systems—into commercially viable and sustainable agricultural solutions.

5.2. Encapsulated Formulations in Agriculture

Prospective Encapsulated Biostimulant Formulations for Different Stress Types

While garlic-based formulations are among the most extensively studied examples of encapsulated plant-derived biostimulants, a growing body of evidence indicates that a wide range of other bioactive sources may be equally or even more suitable for targeted stress mitigation when combined with micro- or nanoencapsulation strategies. The effectiveness of encapsulated biostimulants strongly depends on the type of stress, the dominant mode of action of the bioactive compounds, and the capacity of the carrier system to protect and deliver these molecules under agronomic conditions. An overview of the most promising micro- and nanoencapsulated plant-derived biostimulant formulations targeting different stress conditions, along with their key bioactive components and formulation advantages, is summarized in Table 2.

Table 2. Overview of prospective micro- and nanoencapsulated plant-derived biostimulant formulations for mitigating different stress types in crop protection, highlighting major bioactive sources, dominant mechanisms of action, and the functional advantages of encapsulation.

Stress Type	Promising Biostimulant Sources	Key Bioactive Components	Advantages of Micro-, Nanoencapsulation
Heavy metal stress	Macroalgae extracts; polyphenol-rich plant materials (e.g., tree leaves, agro-industrial by-products)	Polyphenols, flavonoids, polysaccharides	Enhanced stability, improved bioavailability, controlled release, increased metal-chelating and antioxidant activity
Drought stress	Macroalgae; woody plant tissues; osmoprotectant-rich extracts	Betaines, polysaccharides, phytohormone-like compounds	Prolonged activity, improved stress signaling modulation, reduced degradation under field conditions
Herbivory, pest attack	*Allium* species; aromatic and medicinal plants	Sulfur-containing compounds, terpenoids, essential oil components	Reduced volatility, enhanced persistence, controlled release, improved efficacy at lower doses
Oxidative stress (general)	Diverse plant extracts; algae-derived biostimulants	Antioxidants, phenolics, carotenoids	Improved formulation stability, sustained antioxidant delivery, enhanced plant tissue penetration

In the case of heavy metal stress, biostimulants derived from macroalgae and polyphenol-rich plant materials have attracted considerable attention due to their multifunctional protective roles. These extracts are rich in antioxidants, polysaccharides, and phenolic compounds capable of scavenging reactive oxygen species and complexing toxic metal ions. Encapsulation can further enhance these properties by improving the chemical stability of the active components, reducing premature degradation, and enabling more controlled interaction with plant tissues and the rhizosphere, thereby supporting both detoxification processes and stress tolerance [146]. For drought stress, encapsulated biostimulants obtained from macroalgae, woody plant tissues, and osmoprotectant-rich extracts appear particularly promising. These materials often contain compounds that influence phytohormone signaling, osmotic adjustment, and stress-responsive gene expression, contributing to improved water-use efficiency and resilience under water-limited conditions. Encapsulation-based delivery systems offer additional advantages by prolonging the persistence of these bioactives, facilitating gradual release, and reducing the need for repeated applications, which is especially relevant under field conditions characterized by fluctuating environmental stresses [24,143,147]. Herbivory and pest pressure represent another domain in which encapsulated biostimulant formulations may provide substantial benefits. Sulfur-containing compounds, terpenoids, and other volatile or labile secondary metabolites—commonly found in *Allium* species and aromatic or medicinal plants—exhibit well-documented deterrent and antimicrobial activities. However, their practical application is often limited by rapid volatilization and environmental degradation. Micro- and nanoencapsulation can mitigate these constraints by reducing volatility, enhancing persistence on plant surfaces, and enabling more targeted release, thereby improving efficacy while potentially lowering application doses [148–150].

Taken together, these examples underscore that garlic extracts should be regarded as a representative model within a broader and diverse landscape of plant-derived biostimulants suitable for encapsulation. From a prospective viewpoint, the strategic selection of bioactive sources based on stress-specific modes of action, combined with tailored encapsulation technologies, offers a versatile framework for developing next-generation biostimulant formulations aimed at sustainable crop protection.

5.3. Liposomes in Agriculture

The studies discussed in this section demonstrate clear biological activity of liposomes under laboratory conditions; however, evidence for their translation to open-field environments remains limited, particularly for liposome-based formulations.

5.3.1. Translocation Pathways and Stability of Liposomal Nanoformulations in Crop Systems

There are few research results available on the penetration of liposomes. Karny et al., 2018 [151] studied liposomes composed of soy lecithin-derived lipids by applying micronutrients to the surface of tomato plants. After delivery to a single upper leaf surface, the liposomes penetrated the leaf and underwent bidirectional translocation, first to neighbouring leaves and then to other leaves and roots. The liposomes were then internalized by plant cells, where they released their active ingredients within 96 h of treatment. The molar concentration of liposomes applied to the leaf decreased with increasing distance from the treatment point, but the amount required to treat microelement deficiencies remained (100 ppb). A maximum of 33% of the NPs used penetrated the leaf within 72 h, while less than 1% of the free molecules applied in a similar manner did so. The stability of NPs with a short range of 1–2 m was examined after spraying, confirming that the particles collected on the collection plates differed by only 5% from the initial population of pre-sprayed liposomes. In addition, airborne NPs decomposed into safe phospholipids, thus reducing the environmental impact. Studies on the effects of liposome-formulated plant extracts have mostly been conducted under in vitro conditions, with little field data available.

5.3.2. Antiviral Effects of Liposome-Formulated Plant Extracts Against Phytopathogenic Viruses

Liposome-based nanoformulations have been shown to enhance the antiviral efficacy of plant-derived compounds. For example, the flavonol quercetin, known for its antioxidant activity, was extracted from natural bioresources and encapsulated in lecithin liposomes to overcome its inherent poor solubility and instability. When applied to tobacco plants infected with Tobacco mosaic virus (TMV), liposome-formulated quercetin significantly reduced the expression of heat shock protein 70 (HSP70), a host protein facilitating viral replication. The nano-scale size and amphiphilic properties of liposomes enable efficient delivery of quercetin within plant tissues, allowing it to cross cell membranes osmotically and reach intracellular target sites [152].

5.3.3. Antibacterial Effects of Liposome-Formulated Plant Extracts Against Phytopathogenic Bacteria

Liposome-based nanoformulations of plant extracts have shown promising antibacterial activity against several phytopathogenic and foodborne bacteria. A liposome-encapsulated garlic extract, used as a natural biopreservative, exhibited strong inhibitory effects against *E. coli* [102]. Moreover, the combination of garlic extract and nisin, a polycyclic antibacterial peptide, demonstrated a synergistic effect against *Listeria monocytogenes*, *Salmonella enteritidis*, *E. coli*, and *Staphylococcus aureus* [153]. Lecithin liposomes containing allicin—the main active sulfur compound in garlic—displayed high cellular uptake efficiency and enhanced stability, effectively protecting allicin from adverse environmental conditions such as light, heat, and alkalinity. These properties contribute to extended shelf life and reduced odor intensity of the formulations [103].

5.3.4. Antifungal Effects of Liposome-Formulated Plant Extracts Against Phytopathogenic Fungi

There is limited information available on the use of liposome-formulated plant extracts in plant protection, as most studies have focused on their potential applications in the food industry. Investigating the formulation of plant extracts, Gortzi et al., 2017 [153] reported that methanolic and dichloromethane extracts of *Origanum dictamnus*, when encapsulated in liposomes, exhibited enhanced antioxidant and antimicrobial activity compared to the corresponding free extracts. Liposomes containing 1–10% ginger extract were found to inhibit the growth of *Aspergillus flavus* and *Aspergillus parasiticus*, as determined by disk diffusion and microdilution assays [154]. Similarly, liposome-encapsulated ethanolic extracts of *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale*, *Syzygium aromaticum*, and *Laurus nobilis* demonstrated strong antifungal activity against *Aspergillus* and *Penicillium* species [155].

Furthermore, Pinilla et al., 2019 [156] described the antifungal activity of liposome-encapsulated garlic extract against *Penicillium expansum*, *Aspergillus niger*, *Penicillium herquei*, *Fusarium graminearum*, and *Aspergillus flavus* strains in both in vitro and in situ experiments. The formulation effectively inhibited mold growth on bread products for up to five days, confirming its potential as a natural preservative and antifungal agent.

5.3.5. Insecticidal and Repellent Effects of Liposome-Formulated Plant Extracts

Several studies have demonstrated the repellent and insecticidal potential of liposome-formulated plant essential oils and extracts. Liposomal formulations of essential oils from pennyroyal (*Mentha pulegium*), *Ferula gummosa*, and *Baccharis salicifolia* exhibited significant insect-repellent activity [157,158]. Lopez et al., 2020 [119] encapsulated a dichloromethane leaf extract of *Ruta graveolens* into soy lecithin liposomes and chitosan nanostructures using an ethanolic injection method. The nanoformulated extract caused over 70% mortality in *Spodoptera frugiperda* (fall armyworm) Sf9 insect cell cultures, surpassing the insecticidal efficacy of the commercial pesticide chlorpyrifos. The treatment induced chromatin condensation and DNA fragmentation in the affected cells. Release kinetics studies indicated that chitosan nanoemulsions enabled rapid and complete release of the extract, whereas liposomal systems provided a controlled and delayed release profile.

In another study, researchers investigated the insecticidal activity of flavonoid-rich ethanolic extracts from *Tagetes erecta* and *T. patula*, formulated into multilamellar dipalmitoylphosphatidylcholine (DPPC) liposomes. The liposome-encapsulated extracts exhibited high insecticidal activity (98–100%) against *Sitophilus zeamais* larvae within 48–60 h under laboratory conditions [120].

These findings highlight the potential of liposome-based nanoformulations of plant extracts as environmentally friendly alternatives to synthetic insecticides, offering controlled release, enhanced stability, and improved bioactivity. However, their practical application in crop protection remains limited, emphasizing the need for further field-oriented studies to evaluate their efficacy, persistence, and ecological safety under real agricultural conditions.

6. Conclusions

The integration of plant-derived biostimulants with nanotechnology-based delivery systems represents a promising strategy for sustainable crop protection. Plant extracts provide diverse bioactive compounds that can enhance plant growth, stress tolerance, and defense responses; however, their agricultural use is often constrained by limited stability and bioavailability. Liposomal encapsulation addresses these limitations by enabling more efficient delivery, improved stability, and controlled release of bioactive molecules, thereby increasing efficacy at lower application rates.

From a broader perspective, liposome-based biostimulant formulations have the potential to reduce reliance on synthetic agrochemicals and support environmentally friendly crop protection strategies compatible with integrated pest management and organic farming. Although current evidence is largely derived from laboratory and greenhouse studies, these approaches offer a conceptual framework for next-generation biostimulant and biopesticide systems.

At present, most liposomal and nano-biostimulant formulations remain at proof-of-concept or pre-field stages.

Future research should prioritize field-scale validation, formulation optimization, and assessment of long-term agronomic and ecological impacts to facilitate practical implementation in sustainable agriculture.

Future research should prioritize large-scale field trials under diverse agronomic conditions to validate the efficacy and consistency of liposome-based biostimulants beyond controlled environments. In parallel, efforts are needed to optimize formulation stability, cost-effectiveness, and scalable production processes to support industrial manufacturing and widespread agricultural adoption.

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Abbreviations

ABA	abscisic acid
AGE	aqueous garlic extract
APX	ascorbate peroxidase
CAT	catalase
DLS	dynamic light scattering
DHAR	dehydroascorbate reductase
DPPC	dipalmitoylphosphatidylcholine

EFSA	European Food Safety Authority
FPR	Fertilising Products Regulation
GR	dehydroascorbate glutathione reductase
HSP70	heat shock protein 70
MIC	minimum inhibitory concentration
NP	nanoparticle
OLP	osmotin-like protein
POD	peroxidase
PrxQ	peroxiredoxin
PB	plant biostimulant
PEG	polyethylene glycol
ROS	reactive oxygen species
SnRK	SNF1-related protein kinase
SC-CO_2	supercritical carbon dioxide extraction
SOD	superoxide dismutase
TMV	tobacco mosaic virus
TF	transcription factor
TEM	transmission electron microscope

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