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Review

Harnessing the Power of Biostimulants: A Comprehensive Review of Their Role in Enhancing Agricultural Productivity and Sustainability

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Abstract

Biostimulants are becoming essential and complementary tools in modern agriculture, offering an innovative and environmentally friendly approach to enhance crop production and resilience. This review explores biostimulant classification—including humic substances, seaweed extracts, protein hydrolysates, and beneficial microorganisms—and elucidates the complex mechanisms of action through which they modulate plant physiology and biochemical and molecular processes. A major focus is placed on their demonstrated ability to significantly enhance nutrient use efficiency, stimulate root growth, improve water retention, and activate plant defense systems, thereby increasing tolerance to a wide plethora of stressors like drought, salinity, and extreme temperatures. Despite their proven efficacy, the broader adoption of biostimulants faces significant hurdles, including inconsistent product formulation, fragmented regulatory frameworks, and a limited understanding of their long-term effects under diverse field conditions. By emphasizing recent scientific and technological innovations, this review proposes a framework for research and practical application. Finally, biostimulants are part of the new technologies capable of improving plant resistance to abiotic stress and adapting agricultural systems to climate change, thanks to improved productivity and efficiency in the use of production inputs and natural resources. Advances in understanding the mechanisms of action of biostimulants will enable us to modulate the concept of plant nutrition and improve crop management.

Keywords: biostimulants; crop productivity; sustainable agriculture; plant resilience

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1. Introduction

Global agriculture faces numerous challenges, including soil degradation, climate change, and the overuse of chemical fertilizers, which have deleterious effects on the environment, biodiversity, and contribute to global warming, threatening long-term sustainability and crop productivity [1–4]. These environmental and agronomic pressures are

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further intensified by the projected global population increase, which is expected to reach nearly nine billion people in 2050 [5]. In light of these issues, there is an urgent need to identify and develop sustainable compounds capable of enhancing plant growth, nutrient uptake, fruit quality and stress tolerance. Among these compounds, biostimulants stand out as a promising and environmentally friendly alternative to reduce the application of conventional agrochemicals, contributing to more resilient and environmentally friendly farming practices and ensuring food security.

New strategies in plant nutrition, aimed at utilizing biostimulants to enhance the efficiency of natural resource use and production inputs, as well as tolerance to abiotic stress, are of interest in the context of an environmental sustainability approach to crop management. However, to achieve this goal, it will be necessary to define the conceptual framework of biostimulants, their functions, and their benefits in agricultural systems to propose new strategies in plant nutrition.

The term biostimulant has evolved from 1951 to the present (Figure 1), although its use has been imprecise for many years. The comprehensive evolution of this concept is summarized in a table previously reviewed by Yakhin et al. [6]. In 1951, Filatov [7] defined biogenic stimulators as special, non-specific natural substances that stimulate the life reactions of the organism into which they are introduced. It was first clearly defined by Zhang and Schmidt [8] as "materials, other than fertilizers, that promote plant growth when applied in minute quantities". This definition represented a notable advancement over earlier descriptions, since it helped to formally distinguish biostimulants as a separate category of agricultural inputs, distinct from conventional nutrients and agrochemicals.

In 2011, the European Biostimulant Industry Council (EBIC) was formally established. Later, in 2015, du Jardin [9] proposed that biostimulants are substances or microorganisms that stimulate natural plant processes, enhancing nutrient uptake, stress tolerance, and crop quality independently of nutrient content. This definition shifted the focus from product composition to biological function, laying the foundation for a more mechanism-oriented interpretation of biostimulant activity.

The development and dissemination of the biostimulant concept, especially those involving microorganisms, has been heavily influenced by industry stakeholders. Companies operating in this field have taken an active role by forming dedicated organizations such as the EBIC and the Biostimulant Coalition in the United States. These groups engage with policymakers, researchers, and other stakeholders to promote the use of biostimulants in sustainable agriculture, and to provide a unified industry voice to regulators and policymakers, encouraging research, innovation and science-based regulation. Furthermore, the industry has also played a key role in the inclusion of biostimulants as a separate product category in agricultural regulations, fostering academic and public dialogue with respect to the rapidly growing global biostimulant market.

Currently, EU Regulation 2019/1009 [10] recognizes biostimulants as a distinct category of fertilizing products. According to this regulation, a biostimulant is defined as a product that stimulates plant nutrition processes independently of its nutrient content, with the sole purpose of enhancing one or more of the following plant or rhizosphere characteristics: (i) nutrient use efficiency, (ii) tolerance to abiotic stress, (iii) quality traits, or (iv) availability of confined nutrients in the soil or rhizosphere (Figure 1). This marked a turning point, standardizing how biostimulants are classified and placed on the market. According to the argument presented in this proposal, the definition highlights the need for an assessment of the functions of biostimulants, rather than merely knowing the active substances.

Based on this definition, plant biostimulants (PBs) are characterized by their claimed agricultural functions and encompass a variety of naturally derived bioactive substances, such as (i) humic and fulvic acids (HSs); (ii) animal- and plant-based protein hydrolysates (PHs); (iii) seaweed extracts (SEs); (iv) silicon; as well as (v) beneficial microorganisms (PMBs), including arbuscular mycorrhizal fungi and nitrogen-fixing bacteria from the genera *Rhizobium* sp., *Azotobacter* sp., and *Azospirillum* sp. (Figure 1). Additionally, other substances such as sludge-derived substances (SSs) or (vermi)compost have been described in the scientific literature in terms of their biostimulant activity. This proposal demonstrates the depth of use of biostimulants, given the variability in plant responses under different crop management strategies.

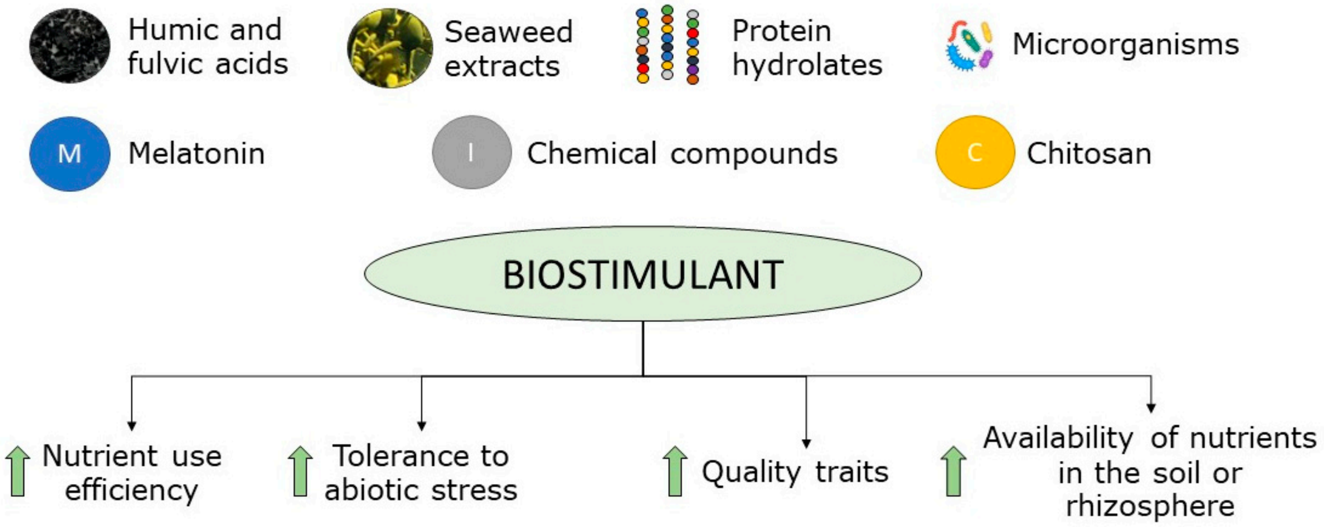

Figure 1. Main groups and effects of biostimulants in crop production.

Biostimulants exert different effects when penetrated into plant tissues through multiple mechanisms that act synergistically to enhance plant development and resilience against a diverse range of abiotic stresses. These mechanisms include stimulation of the metabolism and the nutrient use efficiency, the promotion of root growth through the action of phytohormones such as auxins and cytokinins (CKs), and mobilization of essential nutrients like phosphorus, potassium, and iron via microbial activity and siderophore production [11]. Additionally, some biostimulants induce stress-defense responses by regulating antioxidant machinery, reducing oxidative stress and modulating ethylene production through the enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase, thereby improving plant tolerance to diverse abiotic stresses such as drought, salinity, or extreme temperatures [12]. At the molecular level, active compounds in biostimulants have also been shown to activate signaling pathways that regulate gene expression and enhance systemic induced resistance, coordinately integrating plant growth, nutrition, and defense [6]. However, it should be noted that biostimulants do not include products whose main role is defense against pests and diseases, since for that purpose plant protection or phytosanitary products would be used. This avoids conceptual overlaps between biostimulation, fertilization, and crop protection within crop management.

Based on this background, and recognizing both crop management scientific evidence and persistent variability in agronomic outcomes, this comprehensive review aims to provide a comprehensive synthesis of the current state of knowledge on agricultural biostimulants, with a focus on their mechanisms of action, practical applications, efficacy and future prospects. This work aims to deepen our understanding of the influence of biostimulants on productivity in agricultural systems, based on an understanding of their metabolic functions and their interaction with the agronomic parameters of production quality. We will delve into specific case studies and research findings to illustrate their effectiveness in various cropping systems and environmental conditions. Furthermore, we will discuss the crucial role of biostimulants in advancing agricultural sustainability, examining how their application can lead to reduced fertilizer inputs, improved soil health, and a more resilient and productive food system.

2. Biostimulants Classification

As mentioned above, biostimulants comprise a diverse group of substances and microorganisms that, when applied to plants, seeds, or the rhizosphere, can enhance growth, nutrient uptake, and/or abiotic stress tolerance. Due to their diverse nature, biostimulants are often classified into several main categories based on their origin, composition, and mode of action. A transparent and harmonized classification of biostimulants is a prerequisite for interpreting the wide range of physiological and agronomic responses reported in the literature. The contributions of this classification, based on a comparative study and subject to regulations, demonstrate the evidence of its functions, mechanisms of action, and advantages in crop management.

2.1. Humic Substances (HSs): Humic and Fulvic Acids

Humic and fulvic acids (HAs and FAs, respectively) are natural and complex organic molecules with different molecular weights and solubility obtained from the breakdown of organic matter through microbial activity. HAs are larger humus molecules (generally between 20,000 and 100,000 Daltons) that are soluble in aqueous alkaline solutions and precipitate under acidic conditions, while FAs are smaller (generally between 500 and 2000 Daltons) and more readily absorbed by plants that remain soluble after acidification [13,14]. The influence of HSs on the physical and chemical properties of soil is evident, especially in relation to nutrient element exchange capacity [15], aggregate formation [16], or the processes of soil organic matter mineralization and the stabilization of soil microorganism activity [13]. Nevertheless, HAs have been mainly related to improving soil properties, while FAs directly influence plant metabolism [14]. This functional distinction partly explains why HSs may generate different plant responses depending on soil conditions, plants and application method. Additionally, the physiological effects on the plant are clear because they act as a metabolic and hormonal regulator. This is evidenced by a direct effect on nitrate absorption through the stimulation of H^+-ATPase in roots [17], as well as a positive influence on the elongation and formation of root hairs, mimicking the action of auxins [18]. Thus, as biostimulants, they can be applied to root or foliage and their main role is to enhance plant nutrition and improve overall plant health through several key mechanisms (Figure 2): (i) increasing soil's cation exchange capacity (CEC); (ii) stimulating proton pumps activating membrane H^+-ATPases at the root level; (iii) exhibiting hormone-like effects on plants; (iv) increasing the levels of photosynthetic pigments and antioxidant activity; and (v) modulating plant stress response by enhancing the activity of key enzymes involved in phenylpropanoid metabolism [19]. Nevertheless, further research studies are required to elucidate the effect of humic substances since the response is different depending on their origin, environmental conditions, plant specificity, and dosage and method of application [20]. Concerning their origin, these humic substances can be extracted from organic matter, leonardite, or compost and vermicompost.

In crops such as tomato or potato (*Solanum lycopersicum* L. and *S. tuberosum* L., respectively), the action of humic substances increases photosynthesis and stress tolerance [21,22], possibly as a consequence of the increase in antioxidant concentration [23]. Therefore, HSs exhibit multifunctional performance due to their diverse effects on plant physiology.

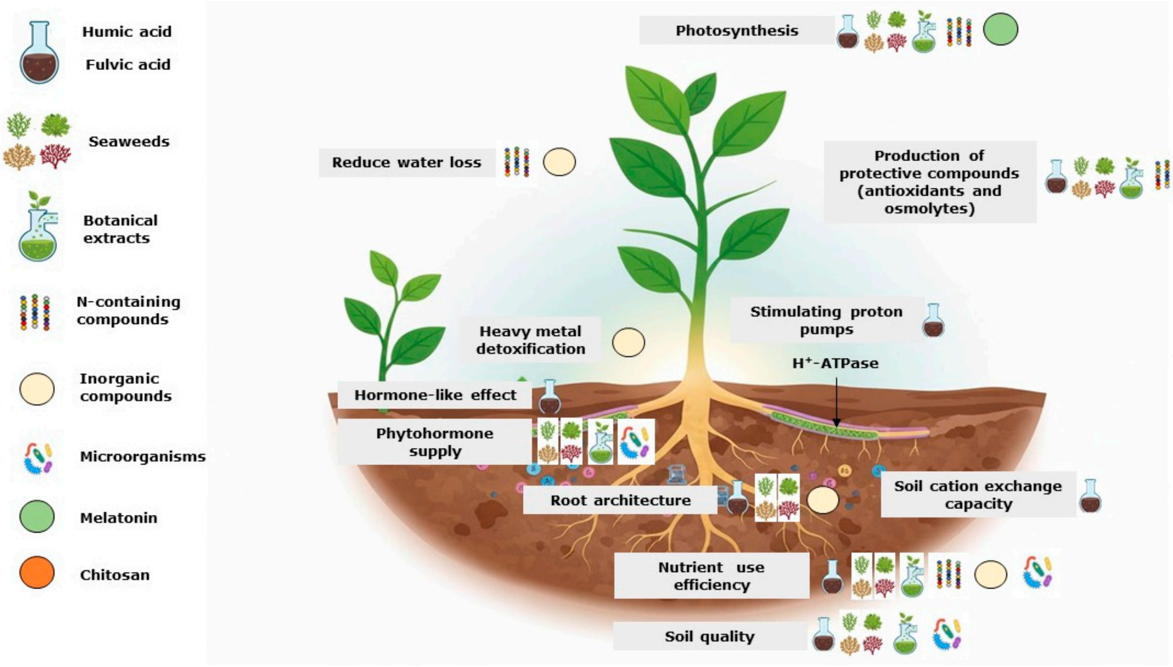

Figure 2. General mechanisms of the different types of biostimulants.

2.2. Seaweed and Botanical Extracts

Derived from various algae and plants, these extracts enhance plant vigor, increase resistance to environmental stress, and improve fruit and flower quality. However, their agronomic performance is highly dependent on botanical origin and extraction procedures, which has driven the development of increasingly complex commercial formulations.

The specific benefits vary depending on the seaweed or plant species from which they are extracted and the extraction method. This has led the research community to develop novel commercial and composite formulations incorporating a mixture of seaweeds or plants and other biological components, including live microorganisms and biopesticides, to improve their efficacy and shelf life.

Seaweeds are considered macroscopic marine algae and are classified into three main classes: *Chlorophyceae* (green algae), *Phaeophyceae* (brown algae) and *Rhodophyceae* (red algae) [24]. These algae constitute a source of micro- and macronutrients, polysaccharides, plant growth regulators, sterols, polyphenols, carotenoids, lipids, and N-containing compounds, which seem to be dependent on the algae species [24,25]. The most predominant phytocompounds are: (i) polysaccharides (e.g., agar, alginic acid, fucoidans, laminaranas, and ulvan); (ii) polyphenols (e.g., eckol, phloroglucinol, and 4-bromophenol); (iii) proteins and amino acids (e.g., taurine, domoic acid, glycine, γ-aminobutyric acid, δ-aminovaleric acid, and laminine); (iv) sterols (e.g., fucosterol, ergosterol, clionasterol, and β-sitosteriol); and (v) carotenes (e.g., astaxanthin, lutein, fucoxanthin, and β-carotene) [24,26–30]. Seaweeds can be applied as extracts (SEs) to roots or as foliar treatment and their main role is to enhance plant nutrition and improve overall plant health through several key mechanisms (Figure 2): (i) direct phytohormone supply, stimulating cell division, elongation and differentiation as well as promoting root and shoot growth; (ii) enhancing nutrient use by increasing water and macro/micronutrients' absorption and improving root architecture; (iii) protecting cells from stress damage and preactivating defense genes; (iv) boosting energy production and essential molecule synthesis by increasing chlorophyll (Chl) rate and content; and (v) improving soil quality by supporting beneficial microorganisms [31–38].

Plant extracts, botanical extracts or phytoextracts are a source of biostimulants in terms of providing beneficial compounds, including macro- and micronutrients, secondary metabolites, vitamins, and growth-promoting hormones. Their significance is especially important in low-input or smallholder farming systems because of their cost-effectiveness and availability. Most importantly, these are cheap, affordable and easily available for smallholder farmers compared to synthetic products. The primary mechanisms of action are (Figure 2): (i) stimulating physiological processes through the increase of photosynthesis, respiration and nucleic acid synthesis; (ii) enhancing nutrient uptake and efficiency by modulating soil microbial community and soil characteristics; (iii) increasing stress tolerance by inducing natural plant defense and triggering the production of protective compounds such as antioxidants and osmolytes; (iv) modulating hormonal and enzymatic activity by mimicking the activity of phytohormones [39–43]. The most relevant point to fully address plant extract obtention is the development of a method that can handle the complexity of plant composition without damaging beneficial and biologically active compounds [44]. Organic solvent extraction with ethanol, acetone or methanol is one effective way to address this because they perfectly isolate organic compounds like flavonoids, phenols, and alkaloids [44]. This variability in composition and extraction underscores the importance of careful interpretation of experimental outcomes involving botanical-based biostimulants.

2.3. N-Containing Compounds

N-containing compounds are substances like protein hydrolysates (PHs) and amino acids, which come from the chemical synthesis of plant proteins, and from both the chemical and enzymatic hydrolysis of animal proteins [45,46]. This group is one of the most widely used categories of biostimulants in agriculture to improve plant growth, nutrient uptake, and stress tolerance, making them a key tool for more sustainable and efficient agriculture. Amino acids are the building blocks of proteins and play a multifaceted role in plant growth and development, acting as powerful regulators and protectants for plants. They directly participate in: (i) enhancing nutrient efficiency via their effect as natural chelating agents or by their availability as an organic nitrogen (N) source; (ii) regulating plant growth, development and overall metabolism by their action as precursors of Chl synthesis and vital plant hormones and enzymes; (iii) increasing a plant's ability to tolerate and recover from abiotic stresses through osmotic regulation, antioxidant action and supporting natural defense mechanisms; and (iv) modifying the activity of soil microorganisms and gene expression, reinforcing resilience against abiotic stresses [6,9,45,47,48]. Their role in vegetable cultivation is noteworthy, as they increase the yield and quality of commercial production under various crop management conditions [45,49]. Regarding crop yield and quality, El-Sanatawy et al. [50] observed that protein hydrolysates increased nitrogen use efficiency and protein concentration in wheat. Similarly, San Bautista et al. [51] noted a positive effect of these substances on rice productivity, increasing grain yield by 13%. In the same crop, Sahoo et al. [52] showed that feather hydrolysates increase nitrogen availability and yield in rice. Therefore, biostimulants containing nitrogenous substances yield satisfactory results under stress conditions, particularly in crops with limited nitrogen inputs due to regulatory restrictions.

2.4. Chemical Compounds

Several chemical elements, such as silicon (Si) or selenium (Se), can also be considered as biostimulants, since they play a pivotal role in improving nutrient efficiency, metabolic regulation and abiotic stress tolerance. Their main activity with respect to plants is: (i) enhancing abiotic stress tolerance through deposition in cell walls, reducing water loss, stimulation of the antioxidant system and regulation of the internal ion balance; (ii) improving nutrient use efficiency by increasing nutrient availability and root growth enhancement; (iii) detoxifying heavy metals [53–56]. Although not traditionally classified as biostimulants, these elements fulfil functional criteria aligned with regulatory definitions when applied at non-nutritive doses to stimulate plant processes. Although traditionally not classified as biostimulants, these elements or other nanoparticles such as zinc (Zn), copper (Cu), and iron (Fe) (classified as nanonutrients or nanobiostimulants) are included among inorganic plant biostimulants when they meet functional criteria aligned with regulatory definitions and are applied in non-nutritive doses to stimulate plant processes [57,58].

2.5. Beneficial Microorganisms

Plant microbial-derived biostimulants (PMBs) are a key category of sustainable agricultural products composed of beneficial microorganisms that, when applied to the seed, plant, or rhizosphere, stimulate natural processes to enhance plant growth, nutrient efficiency, and tolerance to abiotic stress. This category mainly includes plant growth-promoting rhizobacteria (PGPR) that can directly or indirectly promote growth and increase nutrient cycling and stress tolerance [59]; arbuscular mycorrhizal fungi (AMF) that are able to extend the root surface area for water and nutrient uptake and increase stress tolerance [60]; and nitrogen-fixing bacteria that can fix atmospheric nitrogen [61]. Thus, they directly impact: (i) soil structure improvement; (ii) nutrient acquisition (nitrogen fixation, phosphate solubilization, mycorrhizal symbiosis; micronutrient chelation); and (iii) phytostimulation through phytohormone production, induced tolerance, defense mechanism activation and soil structure improvement [59,62].

2.6. Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone first discovered in animals in 1958 but later also found in plants, protozoa, fungi and bacteria [63]. It is synthesized in plants from the amino acid tryptophan in mitochondria, chloroplast and cytoplasm, and it regulates its own synthesis [64]. Even though its consideration as a biostimulant has been controversial in the past, currently the significant and wide variety of positive effects that this hormone induces in plants makes it a strong candidate to be part of this classification. In plants, it has been characterized as playing an important role in seed germination, growth, seedling development, fruit senescence, protection with respect to photosynthesis and stomata, regulation of multiple enzymes, and enhancing the metabolism of multiple secondary metabolites and hormones [64–68]. Additionally, melatonin application has the capacity to improve plant tolerance for a wide spectrum of species and in terms of both abiotic and biotic stresses, mainly with respect to its capacity to improve the antioxidant capacity of plants. For example, rice enhances flood stress after the application of 20–100 µM of melatonin via enzymatic activity improvement and reduction in malondialdehyde (MDA) content [65]; other authors have evaluated the tolerance improvement of *Citrus* sp. species to drought after the application of 50–100 µM of melatonin via the accumulation of different phenolic and flavonoid compounds [68].

2.7. Chitosan

Chitosan (co-polymer of N-acetyl-d-glucosamine and d-glucosamine) is a biopolymer derived from chitin, naturally found in fungal cell walls and arthropod exoskeletons [69]. It has been increasing in popularity since chitin is the second most abundant polysaccharide in the world, it is non-toxic and biodegradable, and it helps the circular economy because it is mainly obtained from marine industry discards of crustacean shells [70]. It is considered a biostimulant compound with multiple applications in agriculture, especially as a natural alternative to chemical protection since its application has antimicrobial properties [71,72]. However, extensive research has also been conducted on plants subjected to several types of abiotic stresses [72]. For example, chitosan can reduce the negative effects of *Botrytis cinerea* in strawberry and grape fruits through the modulation of jasmonic acid (JA) signaling and antioxidant compound production, which reduces hyphae growth [73]. Lipid peroxidation, reactive oxygen species (ROS) content, antioxidant enzymes and proline (Pro) are also optimized when using chitosan as a biostimulant compound to boost salt stress tolerance, as [74] has demonstrated in sorghum seedlings.

In response to the growing need for sustainable agricultural practices, a new wave of biostimulant research is focusing on innovative and unconventional sources. These novel materials, often derived from waste streams and underutilized biomass, offer a way to improve crop productivity while promoting a circular economy. The main emerging sources of biostimulants are: microalgae, agroindustrial by-products such as wastewater and sewage sludge, spent mushroom substrate, compost, and higher plant extracts [41,75–78]. These novel sources are not only expanding the range of available biostimulant products but are also contributing to a more sustainable and resource-efficient agricultural system by valorizing waste and utilizing unconventional materials.

In the following section, the specific mechanisms of action of different biostimulants are described.

3. Mechanisms of Action

As previously stated, biostimulants comprise a highly heterogeneous and dynamic group in which new compounds are continuously added to the big families of biostimulants. However, all share a common goal of enhancing plant growth, crop productivity and resilience to biotic and abiotic stresses. Different compounds may boost specific pathways within plants, operating at a physiological, biochemical or genetic level.

According to the proposed classification, it is necessary to study the functions of biostimulants that cause their main agronomic effects. In this way, it will be possible to deepen the agronomic interpretation of the results after their application under stress conditions.

A primary goal in agriculture is enhancing water and nutrient availability in soils for proper growth of plants. Accordingly, several groups of biostimulants have been reported by different authors as highly suitable for this purpose, particularly under deficit irrigation conditions (Table 1). In this regard, using the correct biostimulant doses has previously been described as improving the formation of stable macro-aggregates and major nutrient solubility, and leading to improved CEC, changes in root morphology to facilitate root penetration in the soil, reduced soil erosion and enhanced interaction between plants and microorganisms, all of which is translated into better water and nutrient uptake and retention capacity [79,80]. Such effects have been described in studies conducted under soil and hydroponic conditions [81,82]. As an example, application of different SEs from *Laminaria* spp. and *A. nodosum* in maize (*Zea mays* L.) has been attributed to improved root characteristics, especially root length, surface and tip number, as well as improved leaf content of calcium (Ca), magnesium (Mg), sulfur (S) and molybdenum (Mo) [83]. *A. nodosum* extracts also modulate aquaporin expression (PIP-1 and PIP-2 families) and activities linked to nutrient uptake transport in tomato plants subjected to optimal and deficit irrigation conditions [84]. The size of HSs is also known to modulate stress tolerance, as [85] has already demonstrated in soybean plants under salt stress; in this context, >30 kDa HSs were found to favor Fe and Zn uptake and reduce sodium (Na) uptake. Using SS-derived biostimulants also promotes the colonization of microbiota linked to urea degradation, nitrate reduction and nitrogen fixation. As demonstrated by [1] in rice (*Oryza sativa* L.), this effect indicates that plants could be more efficient in nutrient uptake, especially nitrogen (N). Drought tolerance is also favored by the colonization of the mycorrhiza species *Rhizophagus intraradices* (Schenck & Sm.) Walker & Schüßler, when used as a biostimulant. This inoculation specifically promotes plant growth and water and nutrient uptake under different types of coal mine spoils [86]. Inoculation of PMBs also promotes Si uptake by the activation of Si transporters *Lsi1* and *Lsi2*, in charge of radial transport of Si in roots [87], *HTK* gene expression transporters in roots and potassium (K) accumulation in leaves and roots under K deprivation [88] in rice. Collectively, these studies highlight that improved water and nutrient acquisition represent one of the most consistent and robust biostimulant-induced responses. However, its magnitude depends strongly on the type of biostimulant, dose, and environmental context.

A central pathway in plant metabolism is photosynthesis, which finally determines carbon (C) assimilation by the plant and growth. In this sense, given the widely described correlation between higher photosynthetic capacity and tolerance to biotic and abiotic stresses [89–91], the use of compounds that stimulate plant photosynthesis is a promising strategy for increasing resilience in present and future crops (Table 1). Even if different biostimulant compounds have been linked to a higher photosynthetic capacity, SEs and PMBs are highlighted in the bibliography. However, current research largely focuses on general photosynthetic processes, such as gas exchange or Chl content [92–94]. These studies often overlook crucial processes linked to photosynthesis improvement, such as mesophyll conductance (g_m), or specific biochemical pathways such as ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) carboxylation activity and xanthophyll cycle pigments. *A. nodosum* extracts have been described as important in the acclimatation to short-term drought stress in *Arabidopsis thaliana* L., which strongly reduces g_s, transpiration rate (E) and ABA-related gene expression after biostimulant application. In opposition, photosynthesis, g_m and Rubisco related genes are decreased, suggesting that limitations of CO_2 entrance are not associated with limitations in diffusion through the mesophyll used by the Rubisco enzyme and with respect to maintaining carbohydrate synthesis. Additionally, plants were more capable of dissipating the excess of energy via non-photochemical quenching (NPQ), PsbS (gene coding for 22 kDa protein of PSII) and the enzyme Violaxanthine de-epoxidase (VDE) [95]. Foliar spray of HSs also boosts photosynthetic parameters in *Chrysanthemum morifolium* RAMAT, such as photosynthesis, maximum quantum efficiency of PSII (Fv/Fm) and the effective photochemical quantum yield of PSII (ΦPSII), as well as improves the lamellae structure of thylakoids [96]. SSs also enhance ATP and NADP(H) content, photosynthesis, Rubisco activity and fluorescence in rice plants [78]. Nevertheless, other authors have stated that positive results in SSs are dose dependent, since high, concentrated doses induce a reduction in gas exchange, fluorescence and Rubisco activity or more accumulation of heavy metals [97,98]. Moreover, even if it is almost a rule that biostimulants improve Chl content, in some studies, non-significant or reduced accumulation were found, as in the case of soybean, broccoli, quinoa or okra [97,99–101]; indeed, the positive effect on pigment accumulation after biostimulant application has been detected is species dependent, as Toscano et al. [102] demonstrated in turnip greens and radish microgreens. It should also be noted that PHs' action is primarily focused on the chloroplast, with their main function being the adjustment and protection of the thylakoid membrane in the light-dependent phase of photosynthesis, thus maintaining photosynthetic efficiency [103–106]. These reported results emphasize that biostimulant-induced improvements in photosynthesis are multifaceted and context-dependent, reinforcing the need to link physiological responses with application strategy and crop-specific traits.

Under normal functioning of biochemical pathways (e.g., photosynthesis), plants use O_2 and accumulate H_2O and ROS, such as O_2^-, OH, H_2O_2 and $^1O_2$. Afterwards, plants use some of them as signaling molecules in different developmental processes like growth, cell cycle, programmed cell death or stress responses, among others. However, most of them are scavenged via enzymatic and non-enzymatic pathways. Under harmful conditions, the ROS generation rate is stronger than its elimination, leading to its accumulation. As a result, ROS inactivates enzymes, induces damage in cellular organelles and destroys membranes, which results in oxidative stress and cell death (further information is available in [107,108]. Biostimulants have been widely proven to reduce such damage via reducing its generation or accumulation (Table 1). Multiple studies have focused their attention on studying modifications in antioxidant enzymatic activities, levels of antioxidant molecules and accumulation of ROS, especially H_2O_2 [84,92,101,109]; further information is provided in Table 1. As an example, SEs boost the activity of antioxidant enzymes (e.g., ascorbate peroxidase or glutathione reductase) and antioxidant compounds (e.g., Pro and glutathione) at the same time that they reduce ROS accumulation ($\cdot O_2^-$, OH· and H_2O_2) and lipid peroxidation in maize after a short-term drought period [110]. Nevertheless, stimulation is variety-dependent, as Fiorentino et al. [101] have already demonstrated in quinoa plants under different salt stress levels. Additionally, previous studies have demonstrated that PHs vary their antioxidant capacity depending on the selected enzyme used for hydrolysis and its concentration in soybean, loach and lentil PHs [111–113]. Nevertheless, the accumulation of antioxidant compounds and modifications in antioxidant enzymes after the application of biostimulant compounds is rarely linked to gene expression levels; therefore, this line of research needs further investigation [109]. This limitation restricts the mechanistic interpretation of antioxidant responses and underscores the need for integrative studies combining biochemical, molecular and physiological analyses.

Modulation of growth and plant tolerance is additionally controlled by several plant hormones, the most studied of which are auxins (particularly indolacetic acid-IAA), gibberellins (GAs), CKs, JA, abscisic acid (ABA), brassinosteroids (BRs) and/or ethylene (Table 1). Within this, biostimulants have been described as having a dual participation. Firstly, these compounds may exhibit a hormone-like activity because their products have biochemical properties similar to plant hormones, thereby simulating their mode of action (it is especially common in the case of auxins, GAs and CKs). Among all biostimulants, PHs, SEs and HSs have been broadly studied as biostimulant compounds with this property. As an example, alfalfa (*Medicago sativa* L.), meat-flour PHs and *Arundo donax* L. HSs have GA- and/or IAA-like activity in maize, tomato and chicory plants via modification of shoot and root length [114,115]. Secondly, they not only act as hormone-like compounds in plants, but they also modify the phytohormone profile accumulation, gene expression or enzyme activity of hormone pathways with respect to synthesis, catabolism or signaling. For example, Luccini et al. [116] demonstrated that application of the commercial PH Trainer® in tomato, an auxin-like hormone, negatively modulates the accumulation of BRs, CKs, JA, ABA and CKs biosynthesis-related compounds, resulting in higher root length. It is important to mention that most of these assays have been tested in in vitro conditions or short-term experiments, in both seeds and small seedlings [117–120]; therefore, further research under real conditions is necessary after determining the chemical composition and their specific effects under controlled conditions to validate such effects, as Almadi et al. [121] have already done with respect to olive trees. In the case of PMBs, microorganisms produce and accumulate phytohormones themselves (e.g., auxins, CKs, GAs and ethylene) to modulate plant development and proper interaction with them [122,123]. In the bacteria *Bacillus cereus* Frankland & Frankland strain D1, genes related to IAA synthesis have been identified, as well as IAA accumulation; its inoculation in *A. thaliana* induces the production of lateral roots and root hairs, as well as heat stress and pathogen tolerance by the expression of hormone-related genes, among others [124]. It has also been described that compounds other than phytohormones are used by microorganisms to communicate with plants and modulate plant hormone profiles, as is the case with the interaction of the fungus *Penicillium aurantiogriseum* Dierckx with *A. thaliana* under in vitro conditions, where volatile organic compounds are emitted in modulating root hair formation and root ethylene and auxin signaling by the FERONIA-RALF22 complex [125]. They also modulate the tolerance to abiotic stresses and production under semi-commercial conditions, as is the case with *Trichoderma harzianum* L. cell-free filtrates in tomato plants, whose tolerance is modulated by the accumulation of CKs, IAA, JA and salicylic acid (SA), as well as related genes such as phospholipase D-a (PLD-a), phenylalanine ammonia lyase (PAL) and IAA-amido synthetase (IAS), among others [126]. The complexity of hormonal regulation mediated by biostimulants, highlighting the importance of distinguishing between direct hormone-like effects and indirect modulation of plant signaling networks, was reported.

Consequently, plants are not axenic organisms but rather harbor a plethora of microorganisms with which they interact in either beneficial or pathogenic interactions, which is nowadays a hot topic in agricultural research. To achieve a successful interaction and communication, it is required that both microorganisms and plants emit a series of signaling molecules, among them hormones, carbohydrates, nutrients, organic acids or volatile organic compounds (further information can be found in [108]). To facilitate such interaction, it is possible to add microorganisms directly to the media as biostimulants, a practice previously shown to improve tolerance to multiple stresses. However, many of these positive effects have been described under in vitro or very controlled conditions [123,127,128]. Under real conditions, it is a common fact that little or no effect may be found after the addition of microorganisms, due to their high competition with other organisms already present in the soil, among other inconveniences [123,129]. To avoid such a situation, it is a common practice to add cell-free microbial filtrates, linked to an improvement of soil microbiome diversity and fruit production under well-irrigated or stress conditions [123,130]. Additionally, other biostimulant compounds, rather than PMBs, are described as improving plant-microbe interactions and microbiota diversity (Table 1). For example, SEs have been linked to the enrichment of the soil, foliar and endophytic microbiome in pepper and tomato plants [131,132]; according to [132], the family *Burkholderiaceae* dominated both the endosphere and phylloplane, and were described as useful for plant growth-promoting bacteria in oil palm and acacias [133]. HSs also promote nodulation between soybean roots and rhizobia via the inhibition of endogenous ethylene accumulation and synthesis genes *Gmmetk*, *GmACS3* and *GmACO* [134]. In SSs, enhancing microbial communities depends on the degree of stability of their components and is dose-dependent due to their toxic effects at high concentrations [135,136].

Therefore, the application of biostimulant compounds is currently a widely used technique that improves plant performance through multiple and diverse mechanisms of action. Among the most studied mechanisms in the present research are the enhancement of nutrient uptake, the boost to photosynthesis, the antioxidant capacity improvement in a plant, the regulation of a plant's hormone profile and the induction of microbiome diversity. The correct use of biostimulants may mitigate current challenges linked to climate change, such as drought, extreme temperatures, or contaminated or degraded soils.

Table 1. Examples of the main biostimulant compounds, including their positive effects after plant application under different environmental conditions. Plant species are also included.

Biostimulant	Species	Stress	Main Effect	Reference
HSs	Wheat	Heavy metal	Improvement of relative water content (RWC), osmotic potential (Ψ_π) and photosynthetic parameters, as well as antioxidant enzyme activities and antioxidant compounds. Reduced accumulation of H_2O_2 and lipid peroxidation.	[137]
HSs	Mugbean	Water and salt stress	Improvement of photosynthetic parameters and antioxidant enzymes. Accumulation of Chl and reduction in membrane damage.	[138]

Table 1. *Cont.*

Biostimulant	Species	Stress	Main Effect	Reference
SEs	Melon	Water stress	Boost to root water uptake and accumulation of antioxidant compounds, such as phenolic compounds and ascorbic acid.	[139]
SEs	*Calotropis procera*	Salt stress	Improvement of photosynthetic parameters and antioxidant enzymes. Accumulation of Chl and reduction in electrolyte leakage. Accumulation of K^+ in relation to Na^+.	[140]
SEs	Tomato	--	Accumulation of proteins and enhanced root development. Improvement of AMF colonization.	[141]
PHs	Tomato	Nutrient deficiency + low temperature	Improvement of root development by accumulation of SA. Accumulation of IAA.	[142]
PHs	Lettuce	--	Alteration of soil microbial communities, stimulating beneficial microbiota and acting as biocontrol for phytopatogens.	[143]
SEs	Soybean	Water stress	Increased water potential (Ψ_W), improvement of photosynthetic and fluorescence parameters, and reduction in Pro accumulation and membrane damage.	[144]
Inorganic compounds	Mango	Water stress	Modification of IAA, CKs, GAs and ABA accumulation in leaves.	[145]
Inorganic compounds	Tomato	Salt stress	Improvement of RWC, leaf turgor potential and photosynthetic parameters (including water use efficiency (WUE)).	[146]
Melatonin	Tomato	Heat stress	Improvement of photosynthetic and fluorescence parameters, as well as relative expression of related genes. Accumulation of Chl and carotenoids, amino acids and sugar. Up-regulation of Chl biosynthesis-related genes.	[66]
Melatonin	Maize	Water stress	Improvement of photosynthesis and accumulation of Chl, ABA, GA_3, IAA and CKs.	[147]
Melatonin	Barley	Low temperature	Enhancement of fluorescence and redox homeostasis, combined with alternation of soil microbiota, especially in groups in charge of N cycling.	[148]
Chitosan	Banana	Low temperature	Accumulation on Chl, phenolic compounds and Pro. Enhancement of antioxidant enzyme activities. Reduced accumulation of oxidative molecules and lipid peroxidation.	[149]
Chitosan	Bentgrass	Salt stress	Enhacement of RWC, Ψ_π, WUE, photosynthesis, fluorescence and antioxidant enzyme activities. Reduction in Na^+ uptake in favor of K, membrane damage and electrolyte leakage. Accumulation of Chl.	[150]
Sludge-derived compounds	Alfalfa	Water stress	Improvement of photosynthetic parameters, RWC, Pro accumulation, and antioxidant enzyme activities. Reduced membrane damage.	[151]
Sludge-derived compounds	Pepper	--	Improvement of nutrient uptake. Accumulation of Chl and carotenoids.	[152]

4. Modes of Biostimulant Application in Agriculture

Plant biostimulants can be applied as seed treatments, through fertigation, as foliar sprays and, more recently, as granular or controlled-release formulations [153]. The correct application method will enable the objectives to be achieved to a greater extent in terms of the plant's physiological response and the effect of the biostimulants. These modes strongly influence both the kinetics and duration of plant responses: seed and soil applications generally provide slower but longer-lasting effects via root system development and rhizosphere modulation, whereas foliar sprays typically induce faster but more transient responses through direct uptake by leaves [154]. The application method should therefore be considered a strategic approach.

Accordingly, application mode should be selected based on the intended agronomic objective (e.g., early establishment, stress mitigation, yield formation or postharvest quality) rather than on product availability alone, an aspect that has often been overlooked in practical recommendations.

Seed priming with PH or SE formulations pre-activates plant metabolism during imbibition and consistently improves germination, seedling vigor and early stress tolerance before emergence in *A. thaliana* and horticultural crops under abiotic stress [155–157]. The benefits of this treatment could persist after its application in the initial stages and ultimately determine final yield, after modifying root growth and modulating physiological response. Root growth and metabolic adaptation in the plant can be improved with plant management under stressful conditions, maintaining its effect throughout much of the crop cycle. In contrast, foliar spraying remains the predominant route for non-microbial biostimulants because it can rapidly modulate photosynthesis, Chl content and antioxidant metabolism, as demonstrated for vegetal PHs and SEs in horticultural crops [158,159].

From a technological standpoint, foliar biostimulant applications are still dominated by tractor-mounted boom or air-blast sprayers. Newly introduced technologies allow for new forms of application adapted to new crop management conditions, particularly through the emergence of unmanned aerial vehicles (UAVs), which enable ultra-low-volume (ULV) applications. These systems allow biostimulants to be sprayed at reduced carrier volumes while maintaining adequate canopy coverage and physiological effectiveness, as recently demonstrated in several field-scale studies [51,160,161].

Beyond foliar spraying, microgranulated fertilizers fortified with biostimulant fractions such as PHs or HSs represent a slower-release delivery mode, localized in the root zone, which has been associated with improved nutrient acquisition and early plant growth [162,163]. In drip-irrigated systems, particularly under arid and semi-arid conditions, fertigation with humic substances or seaweed-derived biostimulants has been linked to enhanced root growth, WUE and yield under water-deficit conditions by acting directly in the rhizosphere [159,164,165]. These root-targeted strategies are particularly effective when the primary objective is to enhance nutrient uptake efficiency or mitigate water and salinity stress.

Recent field evidence further suggests that combining different application modes may outperform single strategies, although responses remain context-dependent. A field study on mung bean confirmed that combining SE seed priming with foliar sprays can outperform single applications in some years, while in others foliar sprays alone deliver similar yield and nutrient-uptake benefits [166]. Consequently, the impact of environmental conditions on different phenological stages of the crop determines the foliar applications of these substances, which should be adjusted to flexible programs rather than pre-established strategies.

Biostimulants are also frequently delivered through fertigation, where the product is injected into drip or micro-sprinkler systems and distributed directly to the root zone. This mode is particularly suitable for HSs, SEs, PMBs and PHs that primarily act on root architecture, rhizosphere chemistry and nutrient uptake, especially under salinity or nutrient-limited conditions [167]; see Section 3 for more information. Thus, it is clear that root growth and emergence are improved, and therefore their tolerance to abiotic stress, when pre-seed treatments are combined with subsequent applications in nutrient solutions or via foliar application [152].

A critical aspect across all application modes is dosage. The agronomic response to biostimulants is strongly dose-dependent, and many studies report non-linear or bell-shaped dose–response curves, where low to moderate rates stimulate growth and physiology, whereas excessive doses reduce performance or even induce phytotoxic effects [168]. Consequently, defining appropriate dose ranges represents one of the main challenges for the effective field deployment of biostimulants. Therefore, knowledge of dose adjustment depending on composition, varietal and species susceptibility, method of application, and environmental conditions is more important than the use of pre-set doses in global contexts. For legume-derived PHs, optimal foliar doses in tomato have been observed at intermediate application rates, with higher concentrations failing to provide additional yield benefits or even depressing photosynthesis and antioxidant balance under stress [109,169]. Similar trends have been reported for SE-based biostimulants and HSs, where supra-optimal concentrations result in diminished or inconsistent responses [170–174].

Therefore, knowledge of dose adjustment depending on composition, varietal and species susceptibility, method of application, and environmental conditions is more important than the use of pre-set doses in global contexts, an approach that aligns well with precision agriculture frameworks and decision-support systems [175,176].

Finally, timing of application relative to crop phenology emerges as a decisive factor. During early vegetative growth and root establishment, soil drench or fertigation with SE-, HS- or PMB-based biostimulants—sometimes combined with early foliar sprays—has been shown to stimulate root system development, canopy expansion and tolerance to water or nutrient stress in several horticultural crops [155,177,178]. Applications around flowering and fruit set are among the most widely adopted in fruit and vegetable crops, where SE-, PH- and mineral-based biostimulants enhance flower retention, fruit set, yield and biochemical quality [179–181]. Preharvest sprays close to harvest often translate into improved firmness, antioxidant capacity, delayed ripening and reduced decay, thereby extending postharvest shelf life [182–184].

Overall, evidence suggests that the agronomic success of biostimulants depends on aligning application mode, dose and timing with the targeted physiological process and the prevailing environmental constraints. In practice, integrated programs combining seed or nursery treatments, root-targeted applications via fertigation, and strategically timed foliar sprays at key phenological stages tend to outperform single-mode strategies. However, these results are valid when the crop and its phenology determine the concentration, dose, and timing of application [185]. The effective integration of modern nutrient application programs into crop management will only be possible based on scientific knowledge that allows adaptation to each growing environment [176,186].

5. Efficacy and Challenges

Despite the broad potential of biostimulants, the agronomic success of biostimulants is highly context-dependent, with crop type emerging as a key factor influencing their efficacy. This context dependency represents one of the critical challenges in extension biostimulant research aimed at achieving consistent field-level outcomes. Legumes and vegetables have consistently demonstrated high responsiveness to biostimulants, particularly to formulations containing PHs, SEs, and PMBs. Studies have demonstrated that common bean (*Phaseolus vulgaris* L.) and tomato exhibit improved nitrogen assimilation, root development, and yield following biostimulant application [187–190]. The use of SEs and moringa extracts, for instance, has been shown to significantly enhance growth and fruit quality in cucumber under greenhouse conditions [191]. These responses are attributed to the short growth cycles and the high metabolic plasticity of legumes and vegetables; in the case of legumes, the response is further supported by their symbiotic nitrogen fixation capabilities. In this sense, crop physiological plasticity appears to be a key determinant of biostimulant responsiveness. In contrast, cereal crops show more variable responses to biostimulants. For example, the application of SE biostimulants in maize enhances drought resilience through modulation of primary and secondary metabolism, although yield gains were only significant under stress conditions [192]. Similarly, despite the fact that the use of microbial inoculants in wheat has improved P solubilization and root colonization, this effect does not consistently translate into higher grain yields, especially under optimal nutrient supply [193]. These inconsistencies may be attributed, in part, to the long history of breeding cereals for yield stability and high performance under intensive agricultural systems, which may have reduced their physiological plasticity and responsiveness to external stimuli such as biostimulants [194]. Fruit crops present unique challenges for biostimulant research and application due to their perennial growth, complex phenological stages, and long production cycles. The article presented by Maanik et al. [195] is a comprehensive overview of the potential of plant biostimulants to mitigate salinity stress in fruit crops, emphasizing their role as a sustainable and environmentally friendly alternative to conventional inputs. Biostimulants enhance salinity tolerance in fruit crops by strengthening antioxidant activity, maintaining ion homeostasis (particularly K^+/Na^+ balance), promoting nutrient uptake, and increasing Chl content and water retention. For example, SEs reduce oxidative damage by improving membrane stability, while microbial biostimulants such as AMF enhance root architecture and nutrient acquisition [196]. These effects collectively enhance plant growth, fruit yield, and quality under salt-stressed conditions in crops like citrus, guava, and pomegranate.

In addition to crop type, the efficacy of biostimulants in enhancing crop performance is strongly influenced by soil type and climatic conditions. Recent studies have shown that the benefits of biostimulant applications are more pronounced in arid, semi-arid, and subtropical/tropical climates compared to temperate regions. This is attributed to the greater environmental stresses in these climates, where biostimulants can play a pivotal role in mitigating adverse effects [197,198]; thus, the concept that biostimulants tend to express their greatest agronomic value when crops are exposed to suboptimal or stress-prone environments. For example, under water-limited conditions, many biostimulant products have been shown to mitigate yield losses in crops, with increases of 25–28% compared with untreated, drought-stressed controls. However, only about half of the evaluated formulations performed consistently across both stressed and well-watered conditions, highlighting variability in efficacy [193]. In contrast, studies on urban plant species exposed to drought have reported limited or inconsistent effects of biostimulant applications. In these cases, growth and biomass were largely unaffected compared to controls, with only minor improvements observed in traits such as height in some woody species. This suggests that the benefits of biostimulants are highly context-dependent, and their effectiveness may be constrained by species-specific physiology, environmental complexity, and site-specific conditions [199]. Several examples of positive, neutral or negative effects are represented in Table 2. Soil characteristics, particularly organic matter content, also play a crucial role in biostimulant effectiveness. Several meta-analyses have consistently shown a decline in biostimulant efficiency with increasing soil organic matter [193,200]. High organic matter levels are often associated with enhanced soil fertility, better water-holding capacity, and a rich microbial community, which may reduce the relative impact of biostimulant applications. Furthermore, the interaction between soil type and biostimulant application methods is critical. For instance, combining seed coatings with soil applications has been demonstrated to significantly increase root biomass, even in soils characterized by low carbon and microbial activity [201]. These results highlight the importance of matching application strategy with soil constraints to maximize biostimulant effectiveness.

Equally critical is the origin and compatibility of microbial biostimulants with the host plant. Different studies emphasize that microbial strains sourced from the same agroecological region or co-evolved with the host crop tend to establish more effective symbioses, resulting in enhanced plant growth and resilience [202,203]. Moreover, some microbial inoculants are highly host-specific. For example, a rhizobia strain effective in one legume species may not perform well in another, and even less in non-legume crops [204]. Microbial biostimulant performance is also influenced by soil microbiome composition. Incompatible or non-native strains may struggle to compete with indigenous microbes, reducing their efficacy [205]. Furthermore, the introduction of microbial inoculants can alter the structure and function of native microbial communities. A meta-analysis revealed that while microbial inoculants can increase soil microbial biomass, they may also induce significant changes in the composition and network complexity of soil microbial communities [206]. In the same way, field studies of AMF inoculants indicate that introduced strains often establish successfully during the initial seasons following application, driven by high propagule density and favorable host–symbiont interactions. However, their relative abundance commonly declines over time as competitive pressures from indigenous AMF and host plant selectivity reassert control. This transient dominance can temporarily alter AMF community composition and colonization patterns, with potential consequences for functional diversity and symbiotic stability once the introduced strains diminish [207]. Moreover, evidence from multi-year studies indicates a decline in efficacy over time, with yield benefits decreasing from approximately 18–20% during the first two seasons to around 13% by the third year. Excessive application frequencies, particularly more than four treatments per growing season, have also been associated with diminishing or even negative effects, possibly due to microbial saturation, shifts in community structure, or adaptive responses in the soil microbiome [193].

To date, most studies have focused on the effects of individual biostimulants, with limited exploration of combined or sequential applications. Understanding these interactions is essential to optimize formulations and application strategies. Research indicates that combining different biostimulants can result in either synergistic or antagonistic effects. For instance, the combined application of biochar and a commercial biostimulant derived from leonardite on pepper plants resulted in synergistic effects of both products [208]. Similarly, integrating microbial biostimulants like *Trichoderma* spp. with PHs has resulted in improved nutrient uptake and stress resilience in several crops [209]. Conversely, other combinations may exhibit antagonistic interactions, diminishing the overall efficacy of the biostimulants. For example, the co-application of biochar and compost in some studies did not yield the expected positive outcomes [210], highlighting the complexity of biostimulant interactions and the need for careful selection and testing of combinations.

While short-term studies have successfully demonstrated the benefits of biostimulants on plant growth and yield, a significant gap remains concerning their long-term effects on soil health, microbial diversity, and nutrient cycling. Some studies have reported that the application of biostimulants can alter soil microbial community structure, potentially enhancing beneficial microbial populations and functions [211]. However, the sustainability of these changes and their implications for soil ecosystem services over extended periods remain underexplored. Furthermore, organic biostimulants such as protein hydrolysates and seaweed extracts can modify soil properties and microbial activity through additions of organic substrates, with effects on microbial community dynamics and nutrient cycling that remain dependent on dose and context [212,213]. Repeated or high-frequency applications may contribute to the accumulation of organic residues and changes in soil C:N balance, with potential implications for intrinsic nutrient mineralization, although mechanistic evidence over multiple seasons is limited [212]. Long-term field trials are therefore essential to assess the persistence of biostimulant-induced changes and to develop evidence-based guidelines that ensure the maintenance of soil health and productivity.

Although biostimulant application has demonstrated measurable benefits in many crops, a major constraint limiting its broader adoption is the lack of standardized definitions, classifications, and testing protocols. Historically, definitions of biostimulants varied widely across countries, often confusing them with fertilizers or plant protection products. To address these challenges, the European Union approved Regulation (EU) 2019/1009 [214], which provides a legal definition of plant biostimulants and establishes harmonized rules for their labeling, safety, and agronomic claims. This regulation distinguishes biostimulants from fertilizers and categorizes them as either microbial or non-microbial. Currently, only four types of microorganisms are approved: *Azotobacter* spp., *Rhizobium* spp., *Azospirillum* spp. and mycorrhizal fungi. This limited list has been a point of contention, as it excludes many beneficial microorganisms, which may hinder innovation and the inclusion of other effective biostimulant agents. Barros-Rodríguez [215] carried out a study in which the regulatory challenges posed by the taxonomic limitations in the EU regulation is discussed. The author suggests alternative methods for evaluating the safety and efficacy of microbial biostimulants, including the use of bioassays on model organisms, to enable a more comprehensive assessment of both potential risks and benefits. Addressing these gaps is critical for fostering innovation, ensuring product efficacy, and supporting the transition to more sustainable agricultural practices. Although the implementation of Regulation (EU) 2019/1009 [214] represents progress toward a unified regulatory framework, at least within the European Union, similar harmonization efforts are still lacking in many regions of the world. Without international alignment, the full potential of biostimulants to support sustainable agriculture remains limited.

Beyond the agronomic and environmental challenges, economic and practical factors also limit the broader use of biostimulants in agriculture. These products are often more expensive than conventional fertilizers or crop protection agents, and their inconsistent performance across different crops, soils, and climates makes it hard for farmers to know whether the investment will pay off [216]. Adoption is further slowed by the lack of clear guidelines and decision-support tools, leaving many growers unsure about which products to choose, how much to apply, or when to apply them to get the best results [217].

Table 2. Examples of biostimulant applications with positive (+) or neutral/negative (0/-) effects.

Biostimulant Type	Concentration	Application	Crop	Stress	Action	Reference
SEs	2.5 L/ha 5.0 L/ha 10.0 L/ha	Soil	Tomato	--	+	[190]
SEs + plant extracts	2.5 L/ha	Soil	Melon	Water stress	+	[139]
SEs	0.5% 1% 1.5%	Foliar	Milkweed	Salt stress	+	[140]
SEs	6.25 mL/L	Soil	Evergreen woody plants Graminoid plants	Water stress	0/-	[199]
HSs	750 mg/L 1000 mg/L	Soil	Wheat	Cadmium toxicity	+	[136]
HSs	100 mg/L	Soil	Rice	Arsenic toxicity	+	[138]
HSs	5 mL/L	Soil	Evergreen woody plants Graminoid plants	Water stress	0/-	[199]
PHs	0.1 g/L 0.2 g/L	Soil/Foliar	Petunia	--	+	[218]
PHs	8 g/L	Soil	Tomato	--	+	[158]
PHs	4 kg/ha	Soil	Tomato	Nutrient deficiency	+	[142]
PHs	5 mL/L	Soil	Evergreen woody plants Graminoid plants	Water stress	0/-	[199]
Silicon	0.3 g/L	Soil	Strawberry	--	+	[219]
Silicon	2.5 mM	Soil	Tomato	Salt stress	+	[146]
Silicon + SEs	1 mM + 10%	Foliar + soil	Sesame	Water stress	+	[220]
PMBs	Not assigned	Soil	Maize	Water stress + Heat stress	+	[221]
PMBs	15 g with soil	Soil	Barley + alfalfa	Water stress	+	[222]
PMBs	Not assigned	Soil	Triticum aestivum	Salt stress	+	[223]
Melatonin	50 µM 100 µM 150 µM	Foliar	Citrus	Water stress	+	[68]
Melatonin	100 µmol/L	Soil	Tomato	Heat stress	+	[224]
Chitosan	0.5 mg/mL	Soil	Sorghum	Salt stress	+	[74]
Chitosan	0.05%	Foliar	Common bean	Salt stress	+	[225]

6. Future Directions and Research Gaps

According to current knowledge about the action of biostimulants, new research and advances in understanding these substances could modify the concept of plant nutrition, extending the action of nutrition to organic substances, including those derived from minerals, due to the metabolic and physiological changes they can induce in plants.

The use of biostimulants in agriculture has been shown to provide multiple benefits that extend beyond conventional plant nutrition. These products represent a key strategy to complement mineral fertilization and advance towards more efficient and sustainable agriculture by reducing dependence on chemical fertilizers. Their application improves nutrient use efficiency, favors microbial activity and soil structure, and in the plant, they regulate physiological and hormonal processes, increasing stress tolerance and photosynthetic efficiency. At the production level, they maintain or increase yields and improve the commercial and nutritional quality of crops, contributing to resilience and sustainability in current agricultural systems.

Despite the evident benefits of biostimulants in improving soil structure, microbial activity, and root absorption, significant knowledge gaps still limit their optimal application in agriculture. For instance, it remains unclear how the specific composition of biostimulants, both microbial and non-microbial ones, interacts with different soil types and crops to enhance nutrient uptake efficiency, crop quality and tolerance to abiotic stresses. Additionally, the relationship between microbial metabolite production, soil aggregate formation, and rhizosphere microbiome modulation requires further study to understand the underlying molecular and physiological mechanisms. The effectiveness of biostimulants is also strongly conditioned by how they are delivered in the field. In particular, UAV-based application often involves very low spray volumes and distinct droplet dynamics, which demand formulation adjustments (e.g., surfactants, humectants, and anti-foam agents) to ensure droplet stability, leaf retention, and biological performance. These gaps limit the ability to design precise and reproducible biostimulation strategies, highlighting the need to investigate how to optimize biostimulant composition and dosage, according to specific agroecological contexts.

However, moving from empirical observation to predictable performance requires closing significant knowledge gaps. The intricate cross-talk between biostimulant components (both microbial and non-microbial) and the specific soil–crop matrix remains under-explored. To unravel these underlying molecular mechanisms, future research must prioritize the integration of omics technologies, including genomics, transcriptomics, and metabolomics. Such high-throughput approaches will allow for a comprehensive understanding of rhizosphere microbiome modulation and the signaling pathways that govern plant–microbe interactions.

The challenge of dose–response reproducibility across diverse agroecological contexts highlights the need for innovative systems. The development of advanced nano-formulations represents a frontier priority; these technologies can enhance the stability of bioactive compounds, ensure targeted release, and improve leaf or root absorption, thereby optimizing biostimulant efficacy under fluctuating field conditions.

Finally, it should be emphasized that biostimulants are expected to become a key component of future crop productivity and of a renewed concept of plant nutrition, grounded in the understanding of metabolic and physiological processes modulated by interactions within the soil–plant–atmosphere continuum, which ultimately governs the dynamic and often unpredictable conditions of crop management.

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Abbreviations

ABA: abscisic acid; ACC: 1-aminocyclopropane-1-carboxylate; AMF: arbuscular mycorrhizal fungi; BRs: brassinosteroids; C: carbon; Ca: calcium; CEC: cation exchange capacity; Chl: chlorophyll; CKs: cytokinins; Cu: copper; E: transpiration rate; EBIC: European Biostimulant Industry Council; FAs: fulvic acids; Fe: iron; Fv/Fm: maximum quantum efficiency of PSII; GAs: gibberellins; g_m: mesophyll conductance; g_s: stomatal conductance; HAs: humic acids; HSs: humic substances; IAA: indolacetic acid; IAS: IAA-amido synthetase; K: potassium; Mg: magnesium; Mo: molybdenum; N: Nitrogen; Na: sodium; JA: jasmonic acid; NPQ: non-photochemical quenching; ΦPSII: effective photochemical quantum yield of PSII; PAL: phenylalanine ammonia lyase; PBs: plant biostimulants; PHs: protein hydrolysates; PGPR: plant growth-promoting bacteria; PLD-a: phospholipase D-a; PMBs: plant microbial-derived biostimulants; ROS: reactive oxygen species; Rubisco: ribulose-1,5-biphosphate carboxylase/oxygenase; RWC: relative water content; Se: selenium; SEs: seaweed extracts; Si: silicon; SSs: sludge-derived substances; VDE: violaxanthine de-epoxidase; Zn: zinc; Ψ_W: water potential; Ψ_π: osmotic potential.

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