Chapter 13
Exogenous Application of Biostimulants and Commercial Utilization

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Abstract Plant biostimulants are specialized goods that are used to boost crop productivity and swiftly spreading throughout the agricultural chemical and seed industries. Biostimulants are distinct from conventional crop inputs like fertilizers or pesticides can influence the plant growth and development through many channels from a single substance for influencing crop growth and development depending on both the timing and the location of application. However, there is wide variation in the effectiveness of biostimulants and little knowledge of the mechanisms underlying situations where variations are seen in field-tested experiments. These unidentified pathways might coincide with established indices of soil health, opening doors to untapped biostimulant potential regarding growth and development of the crop. Therefore, it is most frequently employed to provide the nutrients required to achieve the ideal yield at desired level, regardless of the fertilizer source and mode of administration used. The initial distinction between biostimulants and other agricultural inputs is in the adaptability of particular products in terms of the wanted response. The primary categories of crop biostimulants, known mechanisms of action, instances of their current field efficacy, and a future perspective are all addressed in this chapter.

Keywords Biostimulants · Application method · Yield level · Field efficacy · Soil health

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1 Introduction

Other categories are named for these products as plant biostimulators, probiotics, metabolic enhancers, and biofertilizers (Swift et al. 2018; Garca-Fraile et al. 2017). Plant biostimulants (PBs) are the most popular term used in beneficial manner for crop production. Although biostimulants have been used in commercial agriculture for many years, growers now have access to a much wider range of these compounds. Organic acids, seaweed extracts helpful microorganisms (fungus and bacteria), chitosan, and amino acid or protein hydrolysates are examples of goods that are frequently mentioned as biostimulants (Kauffman et al. 2007; du Jardin 2015; Halpern et al. 2015) and less popular but expanding categories include of concentrated enzymes, charcoal, and microbial extracts. The composition of individual products for the remaining biostimulants, which, more often than not, is not fully known, varies substantially with the exception of concentrated enzymes.

This information gap results from the biological origin of these goods, which contain several constituents. It is anticipated that the product's beneficial activity is caused by synergy among the elements rather than by the individual constituents acting alone (Yakhin et al. 2017; Bulgari et al. 2015). According to Yakhin et al. (2017), synergy among product ingredients makes it challenging to pinpoint the precise processes that trigger a crop response. As a result, a better method for defining biostimulant action should be based on their application and efficacy. Consequently, the remainder of this analysis will concentrate on applied biostimulants' agronomic effects on the production of row crops and their putative connections to soil health and is acknowledged.

Plant biostimulants (PBs) are not considered fertilizers or plant protection products because their main purposes are not to give nutrients or shield plants from pests and pathogens (Rouphael and Colla 2018). These items include elements and/or microbes that improve the availability of nutrients to plant roots and, subsequently, their uptake, promote the plant's ability to absorb nutrients, and, in some cases (Calvo et al. 2014), help the plant adapt to abiotic challenges. Less use of fertilizers is allowed by PBs that are frequently used in agriculture due to their effectiveness in enhancing plant nutrient uptake. Consequently, by potentially reducing the vast amounts of synthetic substances consumed by this activity, this could also help to increase the environmental sustainability of agriculture (Puglia et al. 2021). Starting with basic materials with incredibly various compositions and sources, it is possible to create compounds that have stimulatory effects on plants (Rouphael and Colla 2018). This is why various families of biostimulants have been established, including inorganic salts, complex organic materials, humic and fulvic substances, plant extracts and seaweed, chitosan, chitin derivatives, protein hydrolysates, amino acids and organic acids, animal/vegetable protein, and helpful microorganisms (yeast, filamentous fungi, and microalga bacteria like *Bacillus* and *Azotobacter* spp.) (Du Jardin 2015; La Torre et al. 2016). The beneficial impacts of biostimulants' various components may work in concert to enhance plant growth, productivity, yield, and quality. Because of this, it is still unclear how biostimulants work in general (Koleka et al. 2017). This is why a biostimulant is only considered to be such if it has been shown to boost plant nutrient uptake, production, and resilience to unfavorable environmental circumstances (Yakhin et al. 2017).

2 Role of PBs Coping the Toxic Effect of Compounds

Biostimulants can increase a tolerance of a crop to challenging environmental factors such as drought, UV radiation, high heat, and salinity by applying them sparingly to rhizosphere, plants, and seeds (Du Jardin 2015; Yakhin et al. 2017; Del Buono 2021). The production of chlorophyll and pigments, relative water content, leaf gas exchange or the activity of antioxidant enzymes, which control lipid membrane oxidation and water loss are a few essential biochemical physiological processes which are impacted by salt and drought stress in plants. According to recent studies, PBs may be able to mitigate these damages (Del Buono et al. 2020; Goñi et al. 2018). Furthermore, as biostimulated crops are more effective at getting and utilizing nutrients, PBs may enable a reduction in the usage of artificial fertilizers (Del Buono 2021).

3 Categories of Biostimulant

3.1 Seaweed Extracts

3.1.1 Proposed Mechanisms and Composition

The group of biostimulants known as seaweed extracts is made by processing several types of algae, most frequently macroalgae (seaweeds). Macroalgae are a renewable resource, and the species that are utilized to make the biostimulants (Ugarte et al. 2010) are meticulously watched to enable for continuous harvesting to keep the supply steady. The components of commercial products vary widely, depending on the species used, harvesting stage, and the specific extraction method used by each company (Goñi et al. 2016). Alkaline hydrolysis is the most widely used extraction technique while others include super-critical fluid, water-based, microwave, pressurized liquid extractions' ultrasound, acid hydrolysis, and enzyme (Shukla et al. 2019).

Seaweed extracts include betaines brassinosteroids, polyamines, and plant hormones in addition to the carbohydrates (Stirk et al. 2020). These chemicals work in concert to provide favorable effects in plants, including increased plant growth, resistance to biotic and abiotic stressors, and higher crop quality due to increased nutrient uptake. While it is easy to assess an interest crop response (nutrient absorption, plant growth, grain yield, etc.), it is exceedingly challenging to pinpoint the precise metabolite and mechanism at play in field experiments because of how the environment and agronomic techniques interact. Therefore, crop growth and yield response provide the best indicators of their treatment efficacy.

3.1.2 Efficacy and Field Application

The Roman Columella used seaweed extracts as organic manure amendments to their crops and as mulch throughout the first century, according to historical records (Newton 1951). The alleviation of alterations in environmental conditions regard as abiotic stress which mostly comprises of tolerance of water stress, is the targeted mode of action for seaweed extracts' foliar application of row crops. Additionally, there is a transformed emphasis on soil treatments to improve root development and root zone microbial activity. Phytohormones included in the product, stress reduction, and/or stimulation of plant metabolism have all been linked to increased nutrient uptake, grain yield, and plant growth when seaweed extracts are used (Calvo et al. 2014; González et al. 2013; Craigie 2011; Khan et al. 2009).

However, the absence of issued studies showing decreased yields and growth pattern do not imply that products enhance performance of crops if treated with seaweed extracts. Earlier studies concentrated on the differences between untreated and treated crop plants, and there is little information on how seaweed extract interacts with field conditions and other agronomic strategies.

3.2 Humic and Fulvic Acids

3.2.1 Proposed Mechanisms and Composition

The complex process of microbial breakdown of organic matter results in a wide range of different by-products along the course of degradation (Nardi et al. 2007). The final outcome of this route is traditionally thought to be soil organic matter (SOM), which is made up of refractory components that are not degraded due to resistance and is thought to contain stable chemical compounds collectively known as humus. These molecules are frequently categorized as humic acids (HA), fulvic acids (FA), and humin (acid insoluble and alkali), and the organic matter made in the soils is up to 60%. Humic acids are acid insoluble and alkali soluble (Lamar 2020). Traditional theories hold that these substances have degradation resistance, but a more recent theory holds that dynamically decomposition of organic matter is present and that substances that were once believed to be gone under stable reversible reactions. As a result, these substances have the potential to influence the soil microbiome (Lehmann and Kleber 2015).

3.2.2 Efficacy and Field Application

For many years, HA and FA have been employed as inputs in agricultural production, and their effects on communities of microbes, availability of nutrients, and growth of plants have been thoroughly researched (Celik et al. 2010; Jindo et al. 2016). Depending on the intended purpose, there are many different ways to use HA and FA. The two main uses in row crops are to improve nutrient uptake or amend the soil. It is frequently remarked that the performance of HA and FA treatments for enhancing grain production is not constant even applied in a comparison with common commercial fertilizers.

In addition, there are several accounts of applications of FA or HA at the field level having no advantages or even negative effects (Hartz and Bottoms 2010; de Santiago et al. 2010). Though, FA and HA can improve soil structure, preserve soil ammonium, and have an impact on soil biochemistry that is connected to nitrogen (N) and phosphorus (P) cycling. Alternative viewpoints for the HA and FA market might therefore be best centered on nitrogen management and the potential to enhance soil health.

3.3 Nitrogen-Fixing Bacteria

3.3.1 Known Mechanisms and Common Species

All living microbes require nitrogen, which is crucial for the creation of important substances including proteins and nucleic acids. The biggest source of readily available nitrogen is dinitrogen gas (N_2) in the atmosphere, but only a small number of bacteria (diazotrophs) can use nitrogenase to change N_2 into a bioactive form (NH_3). The metal cofactors of the three different nitrogenase enzyme complexes are vanadium-iron (V-Fe), iron-iron (Fe-Fe), and molybdenum-iron (Mo-Fe) (Zehr et al. 2003). Because not all microorganisms use all three nitrogenases, even if the Mo-Fe cofactor is the most prevalent, N fixation may be hampered by the availability of cofactor minerals (Vitousek and Howarth 1991).

Because oxygen irreversibly inhibits nitrogenase function, bacteria must find ways to safeguard the enzyme from oxygen while they are in aerobic settings. The creation of a heterocyst, which is common to cyanobacteria in aquatic settings, or a nodule are the two most prevalent ways for bacteria to isolate themselves from oxygen (Rhizobia–legume symbiotic association). In order to successfully integrate the diazotroph of interest into an agronomic system and maintain optimal product efficacy and biological nitrogen fixation (BNF), it is essential to understand how the diazotroph defends itself against high-oxygen concentrations.

3.3.2 Efficacy and Field Application

The possible replenishment of N to the growing crop, which reduces the requirement for N to be supplied as fertilizer, is the clear agronomic benefit for the usage of N-fixing microorganisms. Placement of these bacteria close to the growing crop's roots via in-furrow treatments or seed treatment is essential for maximizing their efficacy. In order to ensure a favorable crop response, the use of these bacteria will necessitate recommendations tailored to each particular farm to identify the suitable microorganism with the right administration method and with the necessary agronomic management.

3.4 Phosphorus-Solubilizing Bacteria (PSB)

3.4.1 Known Mechanisms and Common Species

Although P makes up only 0.1% of the water-soluble portion of soil content (w/w), this low availability makes fertilizer P necessary to meet plant need of nutrients for a particular crop yield (Sharma et al. 2013). Phosphorus that is applied to soils might become fixed there, making it unavailable for plant absorption while still contributing to the soil's P reservoir is only up to 90%. Microorganisms primarily achieve the solubilization of inorganic phosphates by the production of organic acids (Kalayu 2019) which can improve P availability in two different ways: (1) by preventing cations like Ca^{2+} (Calcium) and $Fe^{2+}/^{3+}$ (Ferrous/Ferric Iron) from fixing accessible P; and (2) releases mineral P-complexes by lowering the soil pH, particularly Ca (Walpola and Yoon 2012). Through the development of extracellular enzymes, organic phosphates can be hydrolyzed to increase the amount of soil-available P (Tarafdar et al. 2002). Although the mechanisms of P solubilization are well established, there is far less information available regarding the effectiveness of boosting those systems through inoculation or management to improve crop production.

3.4.2 Efficacy and Field Application

Numerous studies have been undertaken on phosphorus-solubilizing bacteria in both natural ecosystems and lab settings over a long period of time (Alori et al. 2017; Saeid et al. 2018). The phosphorus solubilizing microorganisms (PSM) have only lately been introduced as an agronomic input, hence their full commercialization potential has not yet been attained (Kalayu 2019). Because different soil types and agronomic activities (rotation, tillage, and fertilization) affect the amount and the source of soil P differently, it is essential to utilize the right microorganism to maximize P solubilization in the specific system. When three PSM strains were applied, wheat yield increased by 19–24% when compared to an uninoculated control, but by 33% when the three strains were co-applied (Turan et al. 2012). When used in conjunction with other agronomic methods, PSM in agronomic systems clearly has the ability to boost crop P uptake, crop grain yield, and soil-available P, the difficulty is to comprehend the species by environment interactions to maximize their usage. In addition, using PSM to provide P for crop growth rather than fertilizing with external P reduces P contamination of streams and can promote the establishment of more soil microorganisms.

3.5 *Arbuscular Mycorrhizal Fungi (AMF)*

3.5.1 Known Mechanisms and General Morphology

The symbiotic relationships between soil fungus and crop plants are widely recognized, and studies of these relationships have been conducted for a variety of crops, including rice, wheat, maize, and soybean (Mbodj et al. 2018; Sugiyama 2019). Due to the physical characteristics of vesicles and arbuscules that are created by these creatures, endophytic mycorrhizal fungi (AMF) are the most prevalent fungal/plant interaction. According to theory, these fungi coevolved with plant roots to enable adaptability to grow on dry land (Willis et al. 2013).

While genetic analysis enables the isolation and species-level differentiation of bacteria, AMF taxonomy frequently relies on the physical traits of the asexual resting spores. The synergistic link between soil bacteria and AMF and plants is another something that has been better understood as a result of advances in microbiological research, leading to the idea of potential co-inoculation (Miransari 2011). Future crop production will benefit from the understanding that AMF can interact with soil bacteria and impact how biostimulants, which support plant development and soil health, are produced or function.

3.5.2 Efficacy and Field Application

AMF's potential as applied biostimulants has been assessed by a number of research and reviews, with the main functions being to reduce the stress of saline conditions, defend against plant diseases, and boost nutrient availability and absorption, particularly connected to P (Plenchette et al. 2005). Increased uptake of Mg, P, N, Ca, and Kin maize in saline circumstances when AMF were present reduced salt stress (Lee et al. 2015). Additionally, stronger photosynthetic upregulation and a decrease in the generation of and in reaction to ROS were two additional ways that local AMF present alleviated salt stress more than inoculation with foreign species (Estrada et al. 2013).

4 Emerging Biostimulant Categories

4.1 Enzymes

A new class of biostimulants, which are pure enzymes, has been launched with the commercial usage of phosphatases in crop fields. Extracellular enzymes produced by organisms are found in soils; this is particularly evident in plants and microorganisms (Spiers and McGill 1979). These enzymes function as biological catalysts to speed up biochemical reactions, which are sometimes reliant on organic N or P chemicals in soils. Enzymes that can be applied to soil in cropping systems have recently been produced and purified by industrial manufacture of enzymes through microbial fermentation techniques (Nielsen et al. 2007).

Enzymes associated with the carbon (C) cycle are also of interest because, like phosphatases, they can catalyze the breakdown of residues and offer a potential tool for better management in high-organic matter systems like no-till or cover cropping. Hemicellulose and cellulose are two of the enzymes that are used to break down the polymers in plant tissues. These larger polymers become more hydrolyzable by microbial communities when they are broken down into smaller polymers or monomers. This decomposition may set off a series of events that speed up the mineralization of extra nutrients for upcoming crop absorption. The development of a perfect mixture with numerous enzymes targeting a certain organic component's disintegration and the release of a specific nutrient is theoretically possible.

4.2 Biochar

Pyrolysis, which is the thermochemical degradation of a fuel source without the addition of oxygen, produces biochar through high-heat processes (Weber and Quicker 2018). The final product is a highly carbonaceous substance with different properties depending on the source, the processing temperature range, and the processing duration (Leng and Huang 2018). Charcoal, which comes from woody biomass, is one of the most popular types of biochar. Hemicellulose, cellulose, and lignin are the main components of biomass, and when the structures degrade at different temperatures, the biochar's stability and activity change (Yang et al. 2007).

As a fuel source, construction material, filtering method, and most recently as an agricultural soil supplement, biochar is utilized in a wide range of industries. When applied to an agricultural land, biochar is extremely resistant to deterioration and functions as a steady carbon source. It can chelate with soil ions because it is porous and has a wide surface area. Higher plant productivity, improved soil treatment's ability to retain nutrients, and increased water holding capacity are all considered agricultural advantages of biochar (Biederman and Harpole 2013).

However, the potential of biochar as a long-term solution for increased crop production, improved soil health, and increased soil productivity is largely unexplored. Ongoing research into its use in conjunction with good strategies may lead to most direct biostimulant applications that are focused in growing season of plant by boosting the yield and plant growth.

5 The Biostimulant and Soil Health Potential

Numerous factors contribute to soil health, many of which are biologically mediated and can therefore be altered by the use of biostimulants. A few examples of the consistent procedures being developed by the USDA NRCS in collaboration with academic researchers across the United States and soil enzyme activity, total soil organic carbon, and soil respiration rates are considered as soil health evaluation and testing of indicators (USDA-NRCS 2021). These characteristics have the ability to simultaneously affect crop development and soil health since, as was previously said, they can be indications of biostimulant action in row crops. Many farmers use biostimulants in search of a yield response during the growing season, paying less attention to the possibility of long-term effects on their soils and repetitive applications over time.

A biostimulant may not produce a quick effect, but it has the ability to improve the health of the soil over time, increasing harvests in succeeding years. Long-term analyses of the effects of biostimulants on soils are, however, scarce. The possibility for greater carbon sequestration is in addition to a direct effect on soil biological activity for improved soil health.

Long-term studies of bioimpact stimulants on soil carbon soil health and nutrient cycling are therefore necessary. However, the long-term addition of C can modify the C:N ratios of soils, which may trap more N and lower crop performance. Although it might seem like the ideal solution, it will take time and a variety of techniques to fully realize and comprehend the value of using biostimulants to improve agronomic management for long-term improvements in soil health and crop yields.

6 Application Methods and Common Uses

Commercial biostimulants are often first applied to specialty crops since these crops frequently have better potential for profit per acre than row crops (Neill and Morgan 2021). Because specialty crops are often more vulnerable to environmental stressors (Kistner et al. 2018). Since there is no additional expense for application, including the biostimulant application with a current standard management practice gives the product a so-called "free ride." For instance, planting provides the chance to provide biostimulants via in-furrow or seed treatment to all planted acres.

Product compatibility with other agronomic inputs like pesticides and fertilizers is one of the biggest obstacles to the integration of biostimulants into a farmer system. There is a lack of understanding and a need for study defining the potential interactions that may happen during field application because there are so many distinctive chemistries and products on the market. Additionally, the reaction to biostimulants may vary depending on the stage of crop development at the time of application or the interactions with the climate, where extremes in precipitation and temperature may affect crop response.

7 Future Perspectives and Conclusion

The market for biostimulants faces major obstacles because of the countless potential uses for these substances. The intended aim of the application is frequently straightforward, even though all agronomic inputs (such as rotation, fertility, soil amendments, seed genetics, tillage, and pesticides) contain a variety of alternatives for product or method selection. For instance, the four distinct pesticide inputs herbicides, fungicides, insecticides, and nematodes each have a single-intended use that is the eradication of the consistent pest outbreak in form of nematodes, insects, and weeds of other microbial diseases. To stimulate signal pathways for mitigating abiotic stress, foliar spray at vegetative stages is considered to be best, however applying the same biostimulant, such seaweed extract, at planting may alter the microbial communities in the application zone. Growers have a wide range of options on the fertilizer market (Table 13.1).

The initial distinction between biostimulants and other agricultural inputs is in the adaptability of particular products in terms of the desired response. Boosting attention is being paid to increasing grain yield, which is frequently the outcome of more effective nutrient usage, and the primary research method for biostimulant use in row crop systems is now focusing on fertilizer recovery potential. While many biostimulants are intended to be applied to row crops in order to boost production, many products really produce these effects through having an impact on the biology of the root zone and the soil. A more thorough analysis of the impact of biostimulants on biological indicators and soil quality may uncover previously unrecognized advantages of their use. The use of biostimulants as a remedy for more sustainable practices and improved soil quality quantifiable yield increases as a result agronomic strategies are improved for public and governments sectors' awareness and the effects through which water quality is maintained and nutrient management is done.

Table 13.1 Role of plant biostimulants in mitigating heavy metal toxicity

Plant species	PB	Heavy metals	Recommended dose of PB	Results	Reference
Zea maize	Humic substances	Cr	4 mM C HA L^{-1}	Higher biomass production, higher stress signaling, and gene response in transcription, CAT, and proline increases	Canellas et al. (2020)
Zea maize	Silymarin-based biostimulant	Cd	0.24 g L^{-1}	Improves photosynthetic activity with enhanced antioxidant mechanism and expression of gene, restored hormonal homeostasis	Alharby et al. (2021)
Zea maize	Megafol	Metolachlor	2.5 L ha^{-1}	Enzymes (CAT, APX, GPX), lower levels of lipid membrane peroxidation, production, improved antioxidant activities increased germination, biomass	Panfili et al. (2019)
Helianthus annuus	Protein hydrolysates	Imazamox	3 L ha^{-1}	Improved plant growth, enhanced photosynthetic activity, increased chlorophyll contents, and improved stomatal conductance	Balabanova et al. (2016)
Glycine max	Fertiacyl Pòs	Glyphosate	0.4 L ha^{-1}	Slightly low yield losses and little symptoms of chlorosis and necrosis	Constantin et al. (2016)

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