

Review Paper

ENHANCING THERAPEUTIC SECONDARY METABOLITE PRODUCTION BY COMPREHENSIVE BIOSTIMULANT INTEGRATION: HARMONIZING PLANT AND MICROBIAL EXTRACT

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ARTICLE HIGHLIGHTS

- Integrative insights into Non-microbial and Microbial Biostimulants.
- Focus on enhancing significant secondary metabolites and plant quality.
- Comprehensive analysis of sustainable agricultural practices.
- Future scope and positive research directions in Biostimulants.
- Role of biostimulants in stress resilience.

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INTRODUCTION

Biostimulants are substances or microorganisms that, when applied to seeds, plants, or the rhizosphere, stimulate natural processes to enhance nutrient uptake and efficiency, improve tolerance to abiotic stress, and increase crop quality and yield (Chand *et al.* 2024). They eventually support the growth of sustainable agricultural practices, as they are environmentally friendly and are better alternatives to synthetic fertilizers. Biostimulants or plant biostimulants are certain substances, extracts, and microbes that enhance the natural nutritional processes in plants without directly providing nutrients. They are more

ABSTRACT

In recent decades, biostimulants have become recognized as organic alternatives for promoting sustainable agricultural practices, extensively applied across agriculture, horticulture, ornamental plants, and greenhouses. Unlike biofertilizers and biopesticides, biostimulants are derived from plants, animals, and microbes and classified by their chemical composition. They are primarily divided into nonmicrobial and microbial biostimulants. Nonmicrobial biostimulants include humic substances, seaweeds, chitosan, protein hydrolysates, amino acids, and inorganic compounds, while microbial biostimulants consist of plant growth-promoting bacteria and beneficial fungi. This review paper consolidates the recent positive effects of biostimulants on sustainable agriculture and outlines potential research areas for the future. Biostimulants serve as biocontrol agents that promote plant growth and development, also reported to enhance the production of valuable industrial therapeutic secondary metabolites. Traditionally, plants with medicinal properties have been used for natural remedies, and biostimulants aim to elevate the secondary metabolite content in these therapeutic plants. Additionally, the significance of both microbial and Nonmicrobial biostimulants is touched upon. A review of existing literature indicates that biostimulants significantly boost nutrient absorption, enhance resilience against abiotic stress, replenish nutrients, and improve soil quality.

Keywords: biocontrol, biostimulants, microbial biostimulants, nonmicrobial biostimulants, secondary metabolites

related to fertilizers than to most varieties of plant protection products and act in addition to fertilizing products thus reducing the nutrient application rate. Biostimulants are widely categorized as microbial and nonmicrobial biostimulants (Fig. 1).

Recent European Regulation (EU 2019/1009) stated that biostimulant is a fertilizer that aims to enhance plant rhizosphere properties. The goal is not on the nutritional value of the biostimulant itself but on its capability to: a) utilize nutrient efficiency in plants; b) elevate tolerance to abiotic stress; c) improve quality; and d) absorb soil or rhizosphere nutrients (European Regulation 2019/1009). European Biostimulants Industry Council (EBIC)

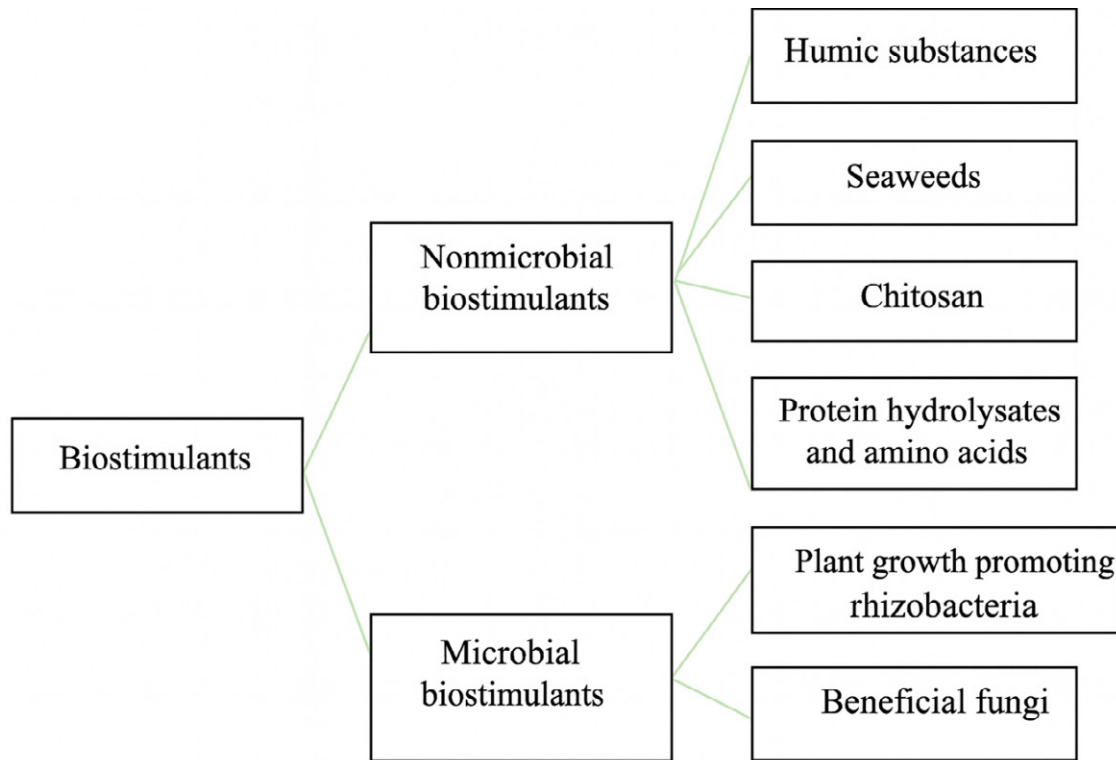


Figure 1 Broad categories of biostimulants

recommended several fundamental principles to standardize and support the claims engineered by producers concerning PB efficiency (European Biostimulants Industry Council 2019).

European Biostimulants Industry Council reported the overall benefits of using biostimulants are: a) stimulating useful compounds in plant roots; b) enhancing plant resistance to environmental abiotic stress factors (flood, drought, and extreme temperature); c) improving soil quality, health, and structure; d) elevating resource availability and decreasing nutrient loss; e) introducing beneficial microbial cultures to aid plants in uptaking and utilizing essential nutrients more effectively; f) protecting plants against opportunistic pathogens and infections; and g) enhancing plant nutrient absorption (Mackiewicz-Walec & Olszewska 2023). Current projects on a European legal framework for plant biostimulants are being established to standardize testing procedures, branding, organizations, and quality control, with full implementation targeted for 2024 (Assi & Zahwan 2023).

The current review systematically integrates the categories and importance of nonmicrobial and microbial biostimulants, focusing on sustainable agriculture practice. The critical view is to

report scientific findings of biostimulants, their significance to boost plant nutrient bioavailability, and enhance plant resistance to both biotic and abiotic stress factors in various economically and therapeutically valuable crops. The comparative analysis of both natural and commercially available biostimulant effects is discussed briefly. In addition, the outline of recent regulations of the European Union (EU 2019/1009) is highlighted.

REVIEW

Nonmicrobial Biostimulants

Humic Substances (Humic and Fulvic Acids)

Humic and fulvic acids are organic compounds found in peat, soil, and other organic materials. Integration between humic and fulvic acids results in the formation of humic complexes (Mackiewicz-Walec & Olszewska 2023). Fulvic acid, an important humic substance derivative of microbial metabolism associated with minerals in soil, supports germination of seeds, encourages plant growth, promotes growth hormones, micronutrient chelation, and retains trace nutrients in the soil and make available to plants. The integrative findings from various papers offer a snapshot that contributes in gaining a

comprehensive understanding of the use of humic substances. Humic acid, one of the types of plant biostimulants is often applied in the rhizosphere region for better utilization (Li *et al.* 2019; Ren *et al.* 2022). Animal matters in soil enhance the degradation and humus formation. On the other hand, humic and fulvic acids showed no significant improvement in promoting nutrient availability, growth, and resistance against stress factors, both biotic and abiotic, suggesting that these acids' effect on plants may depend on environmental conditions and soil quality (Regelink & Koopmans 2021).

The precursor for the degradation of humic acid and its complex identification is a tedious process, due to its low availability in the soil (Canellas *et al.* 2015). Increased crop yield and agro productivity due to the use of humic substances or humic complexes in agriculture leads to the positive effect of humic acid as biostimulants (Regelink & Koopmans 2021). By regulating the architecture of plant roots and shoots involved in nutrient intake, assimilation, and distribution, humic compounds have stimulatory effects. Additionally, humic chemicals can enhance the primary and secondary metabolic pathways linked to plant development, resistance, and abiotic stress tolerance. Humic compounds are biological activators of plant development, and their hydrophobicity, conformational elasticity, and functional group are among the chemical characteristics that substantially influence how effective they are. Numerous European projects, such as BIO-FERTIL (Poland), BIOFEKTOR (Germany), and HUMIC-XL (the Netherlands), have acknowledged the potential application of growth-promoting humic compounds from waste materials (Canellas *et al.* 2015).

Similarly, humic substances, when applied at concentration less than 0.01%, stimulated root growth and root hair elongation upright in *Poa annua* L. by increasing bacterial assimilation in the rhizosphere (White *et al.* 2021). In addition, it is reported that an increase in the assimilation of chosen nutrients supported root expansion, and strengthened drought adaptation in ryegrass cv. Speedgreen upon application of humic acid. The nutrient content of phosphorus, potassium, and zinc in leaf dry weight remains unchanged by humic acid, whereas the increase in iron content was observed (Daneshvar Hakimi Maibodi *et al.* 2015).

Corresponding results demonstrated that the application of humic acid and biomolecules enhanced turfgrasses productivity, but showed different responses to biostimulants applied among creeping bentgrass, tall fescue, and perennial ryegrass (Acuña *et al.* 2022). Likewise, the effect of humic acid-containing fertilizer were evaluated on Duchesne ex-Weston Rozier grown in northern Wielkopolska, Poland, which exhibited leaves with a 60% increase in surface area, intense and dense flowering, higher yield, and more significantly thicker fruit (Zydlik & Zydlik 2023). Furthermore, the application of fulvic and humic acids to sandy and loamy soils with high and extremely low phosphorus levels, respectively, had no effect on grass yield or total nitrogen and phosphorus uptake in fertilized or unfertilized soils. Conflicts arose that the ineffectiveness of the tested humic substances was due to the low doses compared to the soil's existing soil organic carbon levels. Humic substances adsorb to reactive soil mineral surfaces and may not interact with plant roots as effectively as if being put in a hydroculture or soil-less substrate (Regelink & Koopmans 2021).

Seaweeds

Seaweeds (algae or macroalgae) are eco-friendly, nonhazardous, and eco-conscious, being widely used in sustainable and diversified farming. Seaweed extracts encourage germination as well as aid root and shoot maturation. Algae enhance phenylpropanoid pathways in plants and promote root flavonoids and microbe assimilation. Macroalgae-based biostimulant showed improved plant resistance to biotic and abiotic stresses. In 2016, the global biostimulant market was estimated to be worth approximately EUR 1.45 billion, with seaweed extract contributing to this value.

Seaweed biostimulants are abundant in plant hormones, vitamins, minerals, and amino acids. Brown algae, especially *Ascophyllum nodosum*, when applied as organic fertilizer boost crop yield and enhance plant resistance to both biotic and abiotic stresses. Seaweed aids in photosynthetic activity and nutrient uptake with various benefits to improve grass quality during unfavorable conditions (Mackiewicz-Walec & Olszewska 2023). By incorporating perspectives from various literatures the following are formed and a detailed understanding of the differing effects of seaweeds as biostimulants is reported.

Seaweeds are macroalgae, which main groups are classified based on the pigmentation, i.e., Chlorophyta (green), Ochrophyta (brown), and Rhodophyta (red). *Ascophyllum nodosum* contains about 90 groups of chemical molecules, like biologically active components, vitamins, alginic acid, and amino acids. The effects of a mixture containing Bio Algae (commercial brown seaweed derived from *Ascophyllum nodosum*) and mineral fertilizers on mixed stands of forage grasses resulting in an increased yield of 40% (Brouwers *et al.* 2018). The extraction of seaweed biostimulants includes physical (microwaves, pressure, heat) and chemical (solvents, acids, and alkalis) methods influencing the bioavailability of active compounds, determining the efficiency of biostimulants (Kapoor *et al.* 2021).

Worldwide use of seaweed as biostimulant in agriculture has increased its need in global markets. A diverse range of biostimulants, when administered, enhance bioactive compounds in plants and regulate beneficial effects including stress resistance, root shoot elongation, increased chlorophyll content, fruit production augmentation, homogeneous fruit characteristics, senescence retardation, and elevated fruit nutritional quality (Calvo *et al.* 2014). Additionally, Guillard and Inguagiato (2017) reported that seaweed biostimulants had no pivotal impact on Normalized Difference Vegetation Index (NDVI) in regularly manicured turfgrasses (perennial). High-temperature parameters were not included. Hydric stress was considered and periodically monitored, indicating the effect of biostimulants depends on environmental factors. Additional studies were encouraged to determine the seaweed effect on turfgrass (Guillard & Inguagiato 2017).

Chitosan

Chitosan is a polysaccharide containing amino acid groups, composed of polymers of N-acetyl glucosamine and D-glucosamine linked by β -1,4 glycosidic bonds. Chitosan derived from chitin which possesses antifungal properties and aids plants to develop resistance against fungal pathogens. The following reports from existing articles provide a better understanding of chitosan.

Chitin is a complex polysaccharide molecule and an abundant biopolymer in nature, followed by cellulose. Chitin is an environmentally degradable, physiologically compatible and nonhazardous molecule with multiple functional applications (Wolski *et al.* 2019; Goudarzian *et al.*

2020). Due to its potent physiological capacity, the compound chitin has gained increased attention in the last few years. Chitosan application stimulated tyrosine ammonia lyase, polyphenol oxidase, and phenylalanine enzymes, key enzymes involved in plant metabolisms, which furthermore involving antioxidant enzymes like SOD (Superoxide dismutase), catalase, and peroxidase. As biostimulant, chitosan helps plants increase productivity and showed resistance to biotic and abiotic stressors (Wang *et al.* 2017).

Chitin derivatives play a pivotal role in various sectors. The important chitin derivatives are chitosan, N and O sulphated chitin, chitin nanoparticles, alkyl chitin, carboxymethyl chitin, dibutryl chitin, chitin nano-whiskers, chitin nanofibers, chitin nano composites, chitin oligosaccharides, and chitin hydrogels. Chitin and chitosan are reported to positively influence root growth, improve vegetative yield, and improve drought resistance in peppermint (Giglou *et al.* 2022). Furthermore, chitin and chitosan also regulate seed germination and suppress pathogenic fungi wide range of crops (Kanawi *et al.* 2021).

Chitosan promotes grass growth, increases tolerance to environmental stress factors, enhances the defence system, and increased concentration of chitosan (0.1% to 0.2%) leads to leaf elongation and high maintenance. Chitosan, when used in combination with beneficial microbes, improves grass quality and benefits grass management, specifically on golf courses during autumn. Chitosan has a property of moisture sealants when applied as leaf foliar which restricts the use of water and develops resistance against stress factors.

To overcome the negative impacts in agricultural practices, chitosan is used in organic farming. Chitosan forms complexes with toxic metals when applied in soils for biostimulation and botanical remediation. Chitosan regulates abscisic acid signalling pathway and activates light-dependent pathway and guards cell contraction (Hidangmayum *et al.* 2019).

Protein Hydrolysates and Amino Acids

Protein hydrolysates are major groups of plant derived organic fertilizers, consisting of amino acids and peptides or polypeptides. Protein hydrolysates are derived from plant and animal sources by chemical, thermal or enzymatic degradation (Amin *et al.* 2018). According to the Commission Regulation (EU) No.142/2011

(Annex 1, point 14), hydrolyzed proteins such as peptides, polypeptides, amino acids, and their combinations are obtained from the degradation of animal proteins. Biomodulatory effects in protein hydrolysates is due to the presence of carbohydrates, lipids, phytohormones, minerals, phenols, and other organic substances (González-Morales *et al.* 2021). During adverse environmental conditions, protein hydrolysates have been shown to enhance plant adaptability principally by improving antioxidant activity in plant. Chemical traits such as optical activity of amino acids, molecular mass, electrolytic conductivity, and availability of amino acids in protein hydrolysates differ based on the chemical or enzymatic hydrolysis and origin of raw materials, like plant and animal.

Protein hydrolysates promote crop growth and development making them an effective tool for organic agricultural farming. The compounds involved in protein hydrolysates metabolism are not well studied. The effect of protein hydrolysates on soil-habitat microbes in the region is not well known, thereby balancing hydrolysis parameters and enzyme materials during synthesis is critical (Canellas *et al.* 2015). Ugolini *et al.* (2023) studied the effect of protein hydrolysates extracted from oil-free seed cake of Abyssinica kale by two-step enzymatic degradation process. The study of Ugolini *et al.* (2023) also showed that the activity of protein hydrolysates was tested on *Vigna radiate* (mung bean) and *Zea mays* L. (maize). Furthermore, improvement in root development and architecture were reported at 4.8mM of nitrogen concentration corresponding. In addition, protein hydrolysates exhibited high nitrogen content in both root and shoot and also in chlorophyll SPAD index in maize. Most importantly the commercial biostimulant showed the same effect as shown by the natural biostimulant (Ugolini *et al.* 2023).

The application of vegetal protein hydrolysates has gained more interest over the years. They directly stimulate nitrogen and carbon metabolism and indirectly enhance nutrient bioavailability for crop development. In addition, vegetal protein hydrolysates increase the efficient use of nitrogen. Gurav *et al.* (2020) reported the use of protein hydrolysates from animals in brinjal (eggplant) and chili plants, which promoted early blooming and increased yield. Accumulation of ethylene-1-aminocyclopropane-1-carboxylate (ACC) was observed in tomato plants treated with protein hydrolysates from plant source, which also increased the shoot mass (Gurav *et al.* 2020). In

addition, protein hydrolysates obtained from alfalfa upregulated the genes which codes for the component ferredoxin-2, LHCA5 and the enzymes involved in calvin cycle (RuBisCo) (Ertani *et al.* 2017).

Osman *et al.* (2021) studied the effect of three types of protein hydrolysates on foliar application in *Pisum sativum*. The protein hydrolysates NAP (native whey protein), PAH (papain) or PEH (pepsin hydrolyzed whey protein) enhanced the uptake of macronutrients (N, P, and K). Collagen based protein hydrolysates when combined with FeCl₃ exhibited increased Fe content in maize plants in contrast to plants treated with FeCl₃ and FeEDTA individually. ZmTOM1 and ZmIRT 1 gene expression were improved to absorb Fe in roots (Osman *et al.* 2021).

Our review also summarizes other beneficial effects of Nonmicrobial biostimulants on different plants with different modes of application (Table 1).

Microbial Biostimulants

Beneficial Bacteria

Biostimulants, as alternatives to chemical inputs, include useful microbes like growth promoting bacteria and mycorrhizal fungi (Sun *et al.* 2023). Predominantly reviewed bacterial strains are registered subsequently. Plant growth promoting bacteria comprise both rhizobacteria that colonize the rhizosphere and free living bacteria. They play a pivotal role in solubilizing inorganic nutrients and biosynthesis of growth-promoting factors (Baltazar *et al.* 2021).

Biofertilizers are live microbes which play a major role in promoting plant growth. On the other hand, biostimulants are substances that promote plant growth but do not meet plant nutrient demand. The major difference between biofertilizers and biostimulants are that biofertilizers meet the plant nutrients demand and increases crop quality, while biostimulants increase the quality of crop.

Multitude of bacterial genus *Acinetobacter*, *Arthobacter*, *Azosoirillum*, *Enterobacter*, *Ochrobactrum*, *Pseudomonas*, and *Streptomyces* have a wide application in agricultural productivity to facilitate rapid crop growth, enhance nutrient absorption and utilization, minimize nutrient leaching, promote seedling establishment, enhance root growth, remediation of heavy metals-contaminated soil, enhance immune responses,

Table 1 Categories of Nonmicrobial biostimulants and the beneficial effects

Nonmicrobial biostimulant	Plant	Mode of application	Beneficial effect	Reference
Humic substances	<i>Cannabis sativa</i> L.	Fertigation	Observed an increase in cannabinoid content.	(Bernstein <i>et al.</i> 2019)
	<i>Zea mays</i> L.	Foliar	Increased accumulation of trans-zeatin, decreased accumulation of zeatin riboside and positively altered metabolic pathways.	(Gu <i>et al.</i> 2023)
	<i>Oryza sativa</i>	Fertigation	Decreased cell metabolite concentration but increased metabolic marker concentration.	(Canellas <i>et al.</i> 2015)
	<i>Festulolium braunii</i>	Soil treatment	Increase in structural and Nonstructural content except lignin.	(Wiśniewska-Kadzajan & Stefaniak 2020)
Fulvic acid	<i>Rosmarinus officinalis</i> L.	Foliar	Increased fresh and dry yield.	(Farruggia <i>et al.</i> 2024)
	<i>Pisum sativum</i> L. var. <i>meteor</i>	Fertigation	Remarkable increase in pod number, shape, size and seed number per pod, chemical composition of seeds.	(Kamran <i>et al.</i> 2023)
Seaweeds	The sugarcane varieties RB855536, SP803280, and RB855536	Foliar	Increased drought resistance, crop yield, sucrose production and metabolic activities.	(Jacomassi <i>et al.</i> 2022)
	<i>Oryza sativa</i>	Foliar	Increased Ca ⁺ and K ⁺ concentration during drought susceptible condition. Stabilized ROS production.	(Banakar <i>et al.</i> 2022)
	<i>Triticum aestivum</i> L. var. <i>zлата</i>	Fertigation	Higher seaweed content. Increased phenolic content inhibited plant growth but showed increased content of macromolecules, pigments, and secondary metabolites.	(Shibaeva <i>et al.</i> 2023)
	<i>Cucumis sativus</i> L. var. <i>zozulya</i> F1			
	<i>Hyoscyamus niger</i>	Foliar	Enhanced crop growth, leaves yield, and total alkaloids content.	(Al-Taweel & Mohammed 2023)
<i>Ceratonia siliqua</i>	Agar treated with biostimulants	Enhanced plant growth and rooting of carob.	(Zouari <i>et al.</i> 2023)	

Nonmicrobial biostimulant	Plant	Mode of application	Beneficial effect	Reference
Chitosan	<i>Oryza sativa</i>	Foliar	Act as anti-biotic stress factor ultimately increasing the chlorophyll content.	(Banakar <i>et al.</i> 2020)
	<i>Phaseolus vulgaris</i>	Foliar	Increased nutrient uptake efficiency, dry weight biomass and nutrient quality.	(Fawzy <i>et al.</i> 2023)
	<i>Satureja hortensis</i>	Foliar	High concentration of chitosan increased the essential oil content but there is no significant increase in carvacrol.	(Alizadeh <i>et al.</i> 2020)
	<i>Solanum lycopersicum</i> L.	Soil treatment	Showed increase resistance to drought.	(Jiménez-Arias <i>et al.</i> 2023)
	<i>Agrostis stolonifera</i>	Foliar	Increased shoot and root mass, elevated membrane stability under stress condition.	(Li <i>et al.</i> 2022)
Protein hydrolysates and amino acids	<i>Beta vulgaris</i>	Soil treatment	Increased nutrient uptake, altered transcription rate of gene.	(Jolayemi 2023)
	<i>Primula acaulis</i> cv.	Soil treatment	Increased biomass, chlorophyll content and surface area in plants.	(Tütüncü 2024)
	<i>Lactuca sativa</i>	Soil treatment	Successive increase in surface area of leaf, fresh weight of leaf and root and total chlorophyll content.	(Zahra <i>et al.</i> 2024)
	<i>Ipomoea batatas</i> L.	Foliar	Enhanced tuber quality and increased nutrient assimilation.	(Elwaziri <i>et al.</i> 2023)

gives aesthetic appeal to end products, and induce production of plant defence compounds (Cieřlik *et al.* 2022; Nikolouli *et al.* 2021). *Drosophila suzukii*, is an invasive pest species infesting major agricultural soft fruits. *Drosophila suzukii* management is currently based on insecticide applications that bear major concerns regarding their efficiency, safety and environmental sustainability. The sterile insect technique (SIT; Gorrasi *et al.* 2021).

Enterobacter belongs to the family Enterobacteriaceae, under the class Gamma-Proteobacteria. (Nyenje *et al.* 2013). *Enterobacter* spp. are gram-negative, rod-shaped, non-spore-forming, facultative anaerobic bacteria. *Enterobacter roggenskampii* ED5 elicited photosynthetic respiration, plant attributes in sugarcane, and increased overall crop yield (Guo *et al.* 2022). Ullah *et al.* (2020) noted improved kernel grade, Zinc accessibility, yield, and economic viability in Kabuli chickpea by using *Enterobacter* spp. MN17 coating, a Zn-solubilizing endophytic bacterium. In addition, *Enterobacter* had considerable effects on root nodule formation in soybean and maize yield (Ullah *et al.* 2020). *Enterobacter sichuanensis*

AJI 2411, found in the rhizosphere of soybean, improved plant output (Ajibade *et al.* 2023). *Enterobacter cloacae* HG-1 exhibited salinity tolerance and promoted crop development in wheat plants (Ji *et al.* 2020).

Pseudomonas spp. entails abundant gram-negative bacterial strains, which is also the most prolific and diverse bacterial population prevalent in the natural environment (Pramanik *et al.* 2018). Various *Pseudomonas* strains express distinct environment traits involving microbial film formation, fungicidal compound synthesis, symbiotic root adhesion, cell density signaling, chemo attraction, assimilation, exudation, and metabolic dissimilation. *Pseudomonas fluorescens* UM270 decreased salt stress conditions in tomato plants which lead to raise in chlorophyll content, dry weight, and shoot and root extension (Rojas-Solis *et al.* 2023). *Pseudomonas* spp. RGM2987 from Philippine stevia roots exhibited improvement in indole acetic acid, solubilizing phosphate and stress relief enzyme production (Guerra *et al.* 2023). Kumar *et al.* (2021) studied the crop length and fry weight in sunflower using *Pseudomonas lurida*

strain E0026 which results showed an increase in shoot and root length, increase in dry weight, and increase the Cu uptake. *Pseudomonas fluorescens* ECS417 controlled necrosis in leaf developed by *Ralstonia solanacearum* in eucalyptus (Kumar *et al.* 2021).

Streptomyces are filamentous, gram positive, and aerobic bacteria. These bacterial strains are wide producers of secondary metabolites such as antifungals, pathogenic factors, antibiotics, and anticancer agents. *Streptomyces* FJAT-31547 exhibited antimicrobial activities and increased biocontrol performance against *Fusarium* and bacterial rot in tomato plants, due to the presence of n-hexadecanoic acid in the *Streptomyces* strain identified by GC-MS (Zheng *et al.* 2019). In addition, Veilumuthu *et al.* (2022) studied the antimicrobial activity of *Streptomyces* spp. VITGVIOO. *Streptomyces* spp. KRA18-249 exhibited biocontrol activity against various weeds. The secondary metabolite piericidin A secreted by *Streptomyces* spp. FXPO4 controlled fungal growth and inhibited blight damage in potato plants (Veilmuthu *et al.* 2022).

Beneficial Fungi

Microorganisms develop symbiotic relationship with plants, which aids plants in enhancing tolerance to both biotic and abiotic stresses (Zurek *et al.* 2022). Mycorrhizal fungi prevalent in rhizosphere improve crop growth and production. Microbes under this class are called as growth promoting fungal biostimulants (Altaey & Majid 2018). Arbuscular Mycorrhizal Fungi (AMF) improves the carbon flux in subsurface of host plants, which in turn increases the microbial population. This review reports enlighten the importance of beneficial fungi as biostimulants to promote plant yield and growth.

AMF hyphae improves nutrient absorption and translocation expanding from root surface

beyond mineral depletion areas, thus increasing the host plants adaptability (Rouphael *et al.* 2015) Competent microbial strains must exhibit prolonged beneficial effects, are easy to handle, and should not be toxic to environment and mortals (Stingl *et al.* 2022). Xia *et al.* (2021) identified the presence of bacterial (*Pseudomonas veronin* and *Janthinobacterium lividum*) and fungal (*Pseudomymnoascus* spp.) strains predominantly present in endobiome and root microbiome of turfgrasses. These microbes played an important role in bioconversion, nutrient uptake, and pest management (Xia *et al.* 2021).

Giovannini *et al.* (2020) observed the tripartite symbiosis of AMF residing the mycorrhizosphere, plant host, and fungal symbionts. Additionally, AMF support plants fight against pathogen, enhance phosphorous solubilization, improve plant hormones production, function as iron scavengers, and promote antimicrobials synthesis (Giovannini *et al.* 2020).

The work of Caser *et al.* (2019) showed that commercial formulation of *Funneliforms mosseae* and *Rhizophagus intraradices* improved flower yield in saffron, while *R. intraradices* alone increased active metabolites content and radial quenching activity. Schubert *et al.* (2020) industrially produced Micro Tom and Brioso tomatoes using *Rhizoglosum irregularis* and observed increase in free amino acid and carotenoid content up to fourfold when compared to control, while an increase in BRIX values were also reported. *F. mosseae* IMA1 and *Clarideoglosum clarideum* increased ROS scavenging activity and total phenol content, respectively in *Romanesco artichoke* (*C. cardunculus* L. cv. *romanesco C3 Italy* and *Violetto Tema*) (Avio *et al.* 2020).

Other beneficial effects of microbial biostimulants on different plants with different modes of application are presented in Table 2, while beneficial plant traits enhanced by biostimulants are presented in Figure 2.

Table 2 Categories of microbial biostimulants and the beneficial effects

Microbial biostimulant	Plant	Mode of application	Beneficial effect	Reference
<i>Acinetobacter calcoaceticus</i> AC06 and <i>Bacillus amyloliquefaciens</i> BA01	<i>Arachis hypogaea</i> L.	Soil treatment	Observed stress resistance, osmolyte tolerance by altering stress marker activity and physiological redox status in plants.	(Sun <i>et al.</i> 2023)
<i>Bacillus velezensis</i> D2WM (D2), <i>Bacillus velezensis</i> ZJ-11 (ZJ-11)	<i>A. roxburghii</i> (MRH) and <i>A. formosanus</i> (YYB)	Fertigation	Promoted assimilation of nutrients via roots, balancing microbial population in the rhizosphere.	(Shahrajabian <i>et al.</i> 2023)
<i>Bacillus pseudomonas</i> and rhizobium strains	<i>Arabidopsis thaliana</i> and <i>Zea mays</i>	Soil treatment	Upregulated stress-responsive gene, improved crop yield and quality.	(Daengbussadee <i>et al.</i> 2021)
<i>Pseudomonas fluorescens</i> and <i>Burkholderia pyrovocinia</i>	<i>Bracharia brizantha</i> cv.	Soil treatment	Increased photosynthetic limitation by enhancing leaf surface area and increased biomass production.	(Ran <i>et al.</i> 2005)
<i>Trichoderma viride</i> and <i>Pseudomonas fluorescens</i>	<i>Cucurbita pepo</i> L.	Fertigation	Increased content of chlorophylls and carotenoids. Significantly elevated total phenolic profile.	(Bekiesch <i>et al.</i> 2021)
<i>Beauveria bassiana</i>	<i>Triticum aestivum</i>	Seed treatment	Enhanced micro and macro nutrient availability, upregulated phyto-stimulation in plants.	(Sahaja & Pudukarapu 2019)
<i>Aureobasidium pullulans</i> (AK10)	<i>Abies korarua</i>	Seed treatment	Increased tolerance to drought and enhanced microbial colonies.	(Fu <i>et al.</i> 2022)

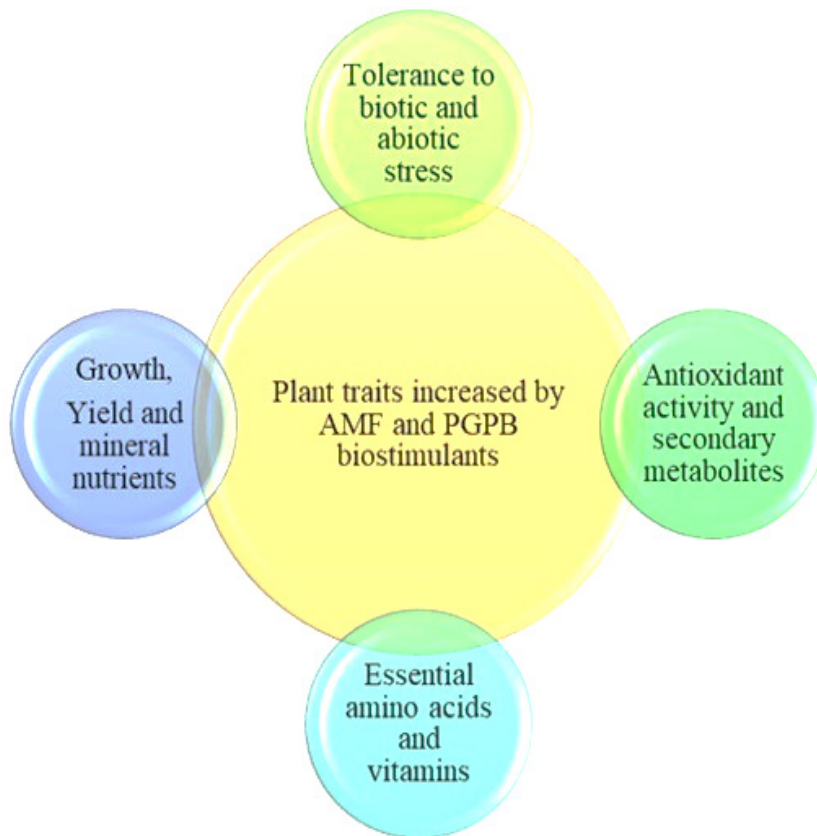


Figure 2 Beneficial plant traits enhanced by biostimulants

CONCLUSION

Both nonmicrobial and microbial biostimulants function differently to enhance sustainable agriculture productivity. Humic and fulvic complexes improve root structure and chelate nutrients; seaweed extracts enriched with bioactive components boost metabolic activity and stress resilience; chitosan primes the plant defense mechanism and acts as a growth regulator; protein hydrolysates directly affect nitrogen metabolism and optimize physiological processes in plants. Simultaneously, microbial biostimulants, including Plant Growth Promoting Rhizobacteria (e.g., *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Enterobacter*, and *Rhizobacter*) and Arbuscular Mycorrhizal Fungi (e.g., Mycorrhizal fungi and *Trichoderma*), improve plant efficiency by forming tripartite symbiotic relationships with plants.

These biostimulants enhance nutrient availability, regulate metabolic pathways, and help plants fight pathogens by modulating root topology, photosynthesis efficiency, and secondary metabolite biosynthesis. These effects improve crop quality and resilience. Notably, the varied effects observed across crops, environmental

conditions, yields, soil types, and application systems emphasize the need for systemized protocols, formulation optimization, and area-specific validation experiments.

The cumulative reports highlight the effective integration of biostimulants in agriculture replacing the use of synthetic fertilizers and conservation of the agriculture land with eco-friendly substances and organisms retaining the soil health and sustainable environment.

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