Soil Studies 13(2), 119-130
http://doi.org/10.21657/soilst.1601789

REVİEW PAPER

Biostimulants for sustainable agriculture in forage crops

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How to cite
Akdağ, N., Sancak, C., & Kahramanoğulları, C.T. (2024). Biostimulants for sustainable agriculture in forage crops. *Soil Studies 13*(2), 119-130. http://doi.org/10.21657/soilst.1601789

Article History
Received 22 August 2024
Accepted 06 November 2024
First Online 28 December 2024

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Keywords
Biostimulants
Forage crops
Sustainable
Nutrient uptake
Stress tolerance

Abstract

Biostimulants, a promising avenue in agriculture, are substances that significantly enhance plant growth and productivity. They are a rich source of various compounds and microorganisms, including humic substances, amino acids, seaweed extracts, chitin and chitosan polymers, inorganic compounds, seed and root extracts, and organic wastes. Humic substances derived from decomposed organic matter are crucial in improving soil structure and nutrient availability. On the other hand, amino acids and protein hydrolysates promote nitrogen uptake and stress resistance, enhancing plant growth. The rich in polysaccharides and phytohormones, seaweed extracts enhance root development and stress tolerance. Polymers such as chitin and chitosan, derived from crustaceans and fungi, provide protective effects against pathogens and environmental stressors. Inorganic compounds and plant extracts also contribute to growth and resistance. The growing global biostimulants market is a testament to the increasing demand for environmentally friendly agricultural solutions, highlighting the urgency of adopting these solutions. Unlike traditional fertilizers, biostimulants do not directly provide nutrients but improve how plants use available nutrients more efficiently. Research underscores the potential of biostimulants to contribute to sustainable agriculture by increasing yield, quality, and disease resistance. Indispensable in modern agriculture, biostimulants are the key to creating sustainable and productive agricultural systems with more resilient plants by stimulating the development of crops, especially under unfavorable conditions, and improving crop quality.

Introduction

Throughout the development of agricultural production, situations such as biotic and abiotic-related stress factors, incorrect and unconscious agricultural practices, excessive fertilization, and irrigation, as well as the use of chemical substances cause a decrease in productivity and quality in the growing areas (Alfosea-Simon et al., 2020; Gürsoy, 2022a). To reduce or eliminate the adverse effects on yield and quality, research is being conducted on applications that regulate plant development and new cultivation techniques. One of these studies is biostimulant applications. Preparations containing organic and inorganic compounds, such as plant nutrients, some growth regulators, seaweed, etc., can be used as biostimulants. Such widespread applications promote plant development, yield, quality, and resistance to abiotic stresses (Şen et al., 2022). Biostimulants are called variously, such as "Biostimulants" or "Plant Activators" (Du Jardin, 2015; Külahtaş and Çokuysal, 2016; Rouphael, 2018). Because of the increasing use of environmentally friendly agricultural products in recent years, research on biostimulant products is increasing daily, and the trade volume is constantly expanding (Povero et al., 2016). The biostimulant market has grown enormously due to the global shift towards sustainable agriculture. Manufacturers are increasingly emphasizing the benefits of integrating biostimulants with conventional fertilizers due to stringent environmental regulations, and ongoing research and development efforts are resulting in innovative formulations that will enhance biostimulant efficacy and meet the increasing demand for eco-friendly solutions. Due to economic and sustainability challenges, various and numerous research studies are ongoing on biostimulants since they are still new in producing traditional agricultural products. Between 2013 and 2022, 77.3% of the research on biostimulants are distributed as research articles, 11.3% as review articles, 5.3% as conference presentations, 4.4% as book chapters, and 1.7% as other research and publications (Anonymous, 2024a).

This review explains research on commonly used biostimulants and some forage crops and their effects on yield and quality.

Application Areas of Commonly Used Biostimulants

For these products, which are used to increase yield and quality, to be included in the biostimulant group, they must have a combined effect on the plant's abiotic and biotic stress conditions (Bulgari et al., 2019). Researchers have various approaches to classifying biostimulants and have listed different compounds in recent studies. The generally accepted classification is as follows; humic substances, amino acids and other nitrogenous compounds, seaweed and plant extracts, chitin and chitosan-like polymers, inorganic compounds, extracts of seeds, leaves and roots (Yakhin et al., 1998, Ertani et al., 2014, Yasmeen et al. 2014, Lucini et al. 2015, Ugolini et al., 2015), organic wastes (Yakhin et al. 2017), beneficial fungi and bacteria (Du Jardin, 2015). Sample studies showing the general properties of these widely used biostimulants and their effects on forage plants are reviewed below.

Humic Substances

Humic substances are natural organic compounds in soil, water, and decomposed plant and animal matter. They are naturally occurring organic substances in the environment, soil, or surface waters. The most commonly used types are Fulvic and humic acids. Fulvic acid is a water-soluble component of humic substances under all pH conditions. Humic acids are the major organic components in soil, forming humus. Humic acid is the most active humus component and is the main compound obtained from soil. They control plant nutrient availability, facilitate carbon and oxygen exchange between soil and atmosphere, and transform toxic chemicals (Piccolo and Spiteller, 2003). Humic substances, used in granular and liquid forms, improve soil physical properties, increase water retention capacity, affect cation exchange and buffering properties, influence nutrient availability, promote transformation of elements for plant use, and increase plant membrane permeability. Humic substances promote the growth of beneficial microorganisms, stimulate plant root systems, and increase hormone production (Lumactud et al., 2022). In a study by Büyükkeskin et al. (2015), it was observed that humic acid application suppressed the growth-inhibiting aluminum toxicity by nearly 50% in Vicia faba L. seedlings under aluminum stress and increased root growth by 21% compared to controls. In addition to this effect, Khaleda et al. (2017) reported that foliar application of a mixture of humic acid and a biostimulant containing catechol and vanillic acid in the growth of annual ryegrass resulted in up to 30% improvement in plant height and green grass yield before and after mowing compared to control plants, as well as about 15% increase in root growth. Furthermore, Shen et al., (2020) examined the effect of humic acid on the physiological and photosynthetic processes of millet seedlings under drought stress. They found that humic acid enhanced seedling growth by improving osmotic regulation, antioxidant capacity and photosynthesis rate, while growth parameters such as plant height, root length and root dry weight improved by 15-29%. Gürsoy, (2022b) investigated the effect of humic acid doses applied as biostimulants on reducing salt stress in sunflower seedlings and compared to control treatments, approximate germination percentage (13.3% increase), average germination time (13.5% decrease), salt tolerance percentage (16. 7% increase), seed length (16.7% increase), root length (25% increase), relative water content (14.3% increase), actual water content (15.4% increase), total chlorophyll (25% increase) and chlorophyll stability index (20% increase) parameters. Makhlouf et al., (2022), who applied humic acid and chitosan to sugar beet plants under severe drought stress conditions, observed that it caused a 1.8% increase in root length, a 4.2% increase in root fresh weight, a 3.5% increase in leaf area, and 4.2% increase in root yield. Alrubaiee and Al-Sulaiman (2023) investigated the effects of different doses of humic acid applied foliarly to oat varieties on herbage yield and some parameters, and reported that green herbage yield increased from 1500 tons per decare in the control application to 2300 tons with an increase of 47%.

Amino Acids and Other Nitrogenous Compounds

This group of biostimulants includes amino acids and peptides derived from plant and animal products. They can enhance plant growth and boost their resistance to stress factors. (Ertani et al., 2009; Malécange et al., 2023). Protein hydrolysates act as plant growth regulators by promoting nitrogen absorption and metabolism in plants (Ryan et al., 2002; Külahtaş and Çokuysal, 2016). They also have indirect effects on plants. When used, these products increase microbial activity in the soil (Du Jardin, 2015). Biostimulants contain amino acids that can be part of plant protein structure. Studies show that certain non-protein amino acids when applied externally, protect plants from stress or activate metabolic signaling (Sharma and Dietz, 2006; Forde and Lea, 2007). Chynoweth and Moot (2013) and Macháč (2013) found that trinexapac-ethyl-based biostimulant increased seed yield by up to 30% on annual and perennial grasses and some forage crops. Przybysz et al. (2014) investigated the effects of Atonik, a nitrophenolate-based biostimulant, on the morphology, physiology, biochemistry and yield parameters of *Medicago truncatula*, which resulted in a 20% increase in chlorophyll content and a 15% increase in protein content. In addition, Trethewey et al. (2016) reported that 400 g/ha trinexapac-ethyl application increased seed yield by 65% on annual grass. Altuner et al. (2019), who applied gibberellic acid pretreatment on the germination of triticale under salt stress, observed a positive increase in germination and growth parameters as the application dose increased. Ciepiela and Godlewska (2019) examined the yield and organic components of Asahi brand biostimulant obtained from three phenolic compounds (sodium para-nitrophenolate, sodium ortho-nitrophenolate, sodium 5-nitroguaiacolate) on Lolium multiflorum at varying nitrogen doses and it was observed that Asahi application at 180 kg/ha nitrogen dose had a positive effect of 40% on yield increase, 28% on chlorophyll content and 22% on protein content compared to control application. Radkowski et al. (2020) studied the effects of a biostimulant containing 18 biologically active free amino acids (L-alpha) obtained by enzymatic hydrolysis on the visual quality and disease and pest resistance properties of perennial ryegrass at doses (1, 2, and 3 l/ha). They reported that visual quality, disease, and pest resistance were positively affected as the dose increased. Again, a biostimulant containing a different plant-derived amino acid was applied to sugar beet leaves by Şanlı et al. (2023). As a result, an increase of approximately 8.5% occurred in the root, stem, and raw sugar yields of the varieties.

Seaweed and Plant Extracts

Seaweed has been utilized as organic matter and fertilizer in agriculture since ancient times. However, only recently have the effects of these products, such as biostimulants in agriculture, begun to be recognized. The presence of polysaccharides, alginates, and carrageenan, as well as their by-products, allows the utilization of seaweed in agriculture. (Külahtaş and Çokuysal, 2016). These extracts aid nutrient uptake, improve soil structure and aeration, and regulate plant growth. Seaweed extracts are considered biostimulants as they improve seed germination, plant growth, stress resistance, and post-harvest shelf life (Mancuso et al., 2006; Rayorath et al., 2008; Khan et al., 2009; Craigie, 2011). In some studies, foliar application of seaweed extracts are associated with increased (about 30%) lateral root development, total root volume, length, and phytohormones such as auxin and cytokinins (Mancuso et al., 2006, W. Khan et al., 2011, Z. Khan et al., 2011). Godlewska and Ciepiela conducted a study on an alfalfa variety in 2018 and applied nitrogen fertilization with seaweed-containing biostimulant and as a result, biostimulants together with nitrogen fertilization increased chlorophyll content by 25%, protein content by 18% and dry matter yield by up to 30%. Godlewska and Ciepiela (2020), in their study on annual ryegrass, found that seaweed extracts decreased NDF content by 10-15%, ADF by 8-12% and ADL by 5-10% compared to the control group, and these results indicate that seaweed extract and amino acid-based biostimulants increase the digestibility of grass plants by reducing their fiber content, while Öner et al. (2023) and Nazzal et al. (2023) reported that plant fresh weight and nutrient uptake from the soil increased by 10%-30% at the macro and microelement levels. This study shows that seaweed extract applied at different doses increases the power of P, K, Ca, Mg, Fe, Cu, Mn, Zn, and B nutrients in alfalfa and in annual ryegrass plants. In addition, Gibson et al. (2024), who applied seaweed-based biostimulant on corn stubble, reported a 24% increase in grain yield and a 30% increase in silage yield in corn planted in the same area the following year. Kaya et al. (2024) examined the effects of different doses of liquid seaweed of organic origin on seed germination and root and shoot growth in some wheatgrass species and as a result, in the germination study carried out after soaking in liquid seaweed solution, it was observed that treatments at 1000 and 2000 ppm doses increased the number of germinated seeds, root dry weight and shoot dry weight by 10% to 35%. Kaya et al. (2024) examined the effects of different doses of liquid seaweed of organic origin on seed germination and root and shoot growth in some wheatgrass species and as a result, in the germination study carried out after soaking in liquid seaweed solution, it was observed that treatments at 1000 and 2000 ppm doses increased the number of germinated seeds, root dry weight and shoot dry weight by 10% to 35%.

Chitin and Chitosan-like Polymers

Chitin and chitosan biopolymers are derived from seafood and mushrooms and are used in food, cosmetics, medicine, and agriculture. Some studies have observed the positive effects of chitin and chitosan on plant physiology. These effects include the impact of their ionic structures on DNA, plasma membrane, cell wall, cell parts, stress factors, and the activation of related genes (Hadwiger, 2013; Katiyar and Singh, 2015). The positive effects of chitosan, such as protection from fungal pathogens, resistance to abiotic stress, and improved fruit quality, are increasing daily. Cho et al. (2007) in a study on sunflower seedlings grown at 20°C for 6 days after soaking in 0.5% and 0.5% lactic acid solution for 18 hours, reported that total weight increased by 12.9% and germination rate increased by 16% compared to the control group. Choudhary et al. (2017) observed that maize plants treated with Cu-chitosan nanoparticles exhibited a 20-30% increase in antioxidant and defense enzyme activities. Additionally, they reported a 15% increase in plant height in pot experiments and a 25% increase in grain yield in field trials. Chitosan applications increase abscisic acid levels 3- times, decreasing stomatal conductance by 40% and transpiration rate by 30%, causing stomatal closure in plants and helping to develop defense mechanisms against environmental stress factors (Iriti et al., 2009). A new slow-release chitosan-silicon nano-fertilizer (CS-Si NF) specially designed by Kumaraswamy et al. (2021) has promoted growth and yield in corn plants. Seeds coated with CS-Si NF at different concentrations had a 43.4% higher yield, with the seedling vitality index increasing by 3.7 times. Jabeen and Ahmad (2013) in safflower and sunflower, Öner and Cengiz (2023) in maize reported that seed coating with chitosan solutions increased germination rate by 20%, shortened germination time by 15%, increased germination index and root number by 25%, and increased root length and coleoptile length parameters by 15%. In another study, Makhlouf et al. (2022) observed that chitosan application to sugar beet plants under severe drought stress conditions caused a 1.8% increase in root length, a 4.2% increase in root fresh weight, a 3.5% increase in leaf area, and a 4.2% increase in root yield.

Seed, Leaf, and Root Extracts

This biostimulant group is obtained from seeds, leaves, and roots extracts. It is obtained chiefly from higher plants such as *Amaryllidaceae, Brassicaceae, Ericaceae, Fabaceae, Fagaceae, Plantaginaceae, Poaceae, Rosaceae, Solanaceae, Theaceae, Vitaceae* and the biostimulants in this group give positive results in sustainable agriculture in plant growth and development, yield and quality and in combating diseases (Parrado et al., 2008; Pretorius, 2013). As a result of the use of extracts obtained from new shoots of some plants as biostimulants, it has been found that it positively affects alcohol degree, pH, total acidity, volatile acidity, color intensity, variable aroma potential index, phenolic compounds, and yield (Sánchez-Gómez et al., 2016). In a study where an aqueous extract obtained from duckweed (*Lemna minor* L.) was evaluated as a biostimulant in corn, corn seeds were coated with different concentrations of this extract (0.01%, 0.05%, 0.50%, and 1.00%). It improved corn germination, biomass (20%), leaf area (25%), pigment content (18%), and vitality index and stimulated nitrogen (22%), phosphorus (19%), potassium (17%), calcium (15%), magnesium (13%), sodium (16%), iron (16%), and copper (%12) interactions (Buono et al., 2021). Similarly, in a study on corn plants under salinity conditions, Prilo et al. (2024) found that duckweed (Lemna minor L.) extract increased biomass (18-22%), root development (15-20%), photosynthetic pigment (25-30%), and soluble protein levels (20-28%). Umarusman et al. (2019) investigated the antibacterial properties of 34 different plant extracts against the pathogen called *Pseudomonas syringae*, which causes leaf blight in people, applied these extracts to seeds. The pathogen suppression rate was revealed by pot and field experiments, and it was stated that the most effective seeded Clove (*Syzygium aromaticum*) extract prevented the disease by 95% in the pot experiment and 98% in the field experiment. Akdağ and Avcı (2023) investigated the seed yield rates of Italian ryegrass (*Lolium multiflorum* L.) at different planting times and biostimulant (Pi-NFS) doses (0, 100, 250, 500 ml da^{-1}). The highest amount of seed was obtained from the 500 ml da^{-1} dose at the 2nd planting time, while the values obtained from the 250 and 500 ml da^{-1} doses at the 1st planting time had 30% higher seed yield values than the control treatments. As a result of a study conducted by Han et al. on the effects of Polygonum minus extract on maize plants under drought conditions, it was reported that the application of the extract increased the wet and dry weight of maize plants by 33.1-41.4% and 48.0-43.1%, respectively, while increasing the chlorophyll b content by 87.9-100.76%, soluble sugar and protein levels by 23.6-49.3% and 48.6-56.9%, respectively. In a study conducted by Peñas-Corte et al. (2024), application of Lamiales plant extract significantly increased maize growth, increasing plant height by 20.45% and yield by 45.67%, while reducing fumonisin concentrations and improving stress tolerance.

Organic Wastes

Some researchers have included food waste or industrial waste streams, composts and compost extracts, fertilizers, vermicompost, wastewater, and sewage treatments in the biostimulant group (Yakhin et al., 2017). Agricultural organic wastes are divided into three groups: Wastes remaining due to plant production, plant mass occurring in cultivated land, forests, fallow land, and fruit and vegetable cultivated areas that cannot be characterized as a product. Stems, straws, shells, seeds, pruning residues, animal manure, and internal organs from slaughter are all included in this group. Animal manure is used as fuel (dung) and fertilizer. Waste from internal organs can also be used as compost fertilizer, and agricultural product processing results in waste. These wastes result from the processes of agricultural products (grinding, sorting, drying, etc.) before being used directly. These are unused wastes such as stems, straws, shells, and seeds. Understanding the effects of post-processing waste on soil properties is crucial for successful recycling efforts. The material obtained after processing is known as biochar, and it is used as a growing medium, silage additive, in poultry feeds, in food or fabric paint, a feed additive, in the cosmetic industry, aromatherapy, and as fuel from pruning residues (Bekar, 2016). Ferreira et al. (2018) reported that a biostimulant application originating from fish waste in second-crop corn plants would be effective on seed yield (18% higher compared to the control treatments), while Qiu et al. (2020) coated meadow clover and perennial ryegrass seeds with different combinations of soybean meal, diatomaceous earth, micronized earthworm compost and concentrated earthworm compost extract as biostimulants and reported that germination rate increased by 5-10%, seedling height by 12-15%, dry weight by 10-15%, while coatings containing soybean meal increased coating integrity by 20% and extended the dispersal time of coatings by 25% compared to uncoated plants. Godlewska and Becher, who used organic wastes consisting of sewage sludge and coal ash as biostimulants, examined some macroelements in *Dactylis glomerata* and *Zea mays* plants in their research in 2021 and decreased cocksfoot calcium content by 15%, magnesium content by 10%, but increased potassium content by 20%. In maize, it decreased calcium content by 12%, magnesium content by 8% and increased potassium content by 18%. These results indicated that the use of waste materials as agricultural fertilizer can reduce the use of mineral fertilizers and can be a suitable method for sustainable agriculture. In addition, Demiray and Parlak (2023) used farmyard manure (3000 kg da^{-1}), chicken manure (300 kg da^{-1}), leonardite (100 kg da^{-1}), They investigated the effects of biological fertilizer (free-living nitrogen bacteria) and chemical fertilizer (10 kg N da^{-1}) applications on the yield and quality of annual ryegrass and reported that while farm manure increased the green and dry grass yield of annual ryegrass by 35%-30%, chicken manure and leonardite increased the yield by 28-24% and 22-20%, respectively. In another study, Saadat et al. (2023) surveyed flowering and yield parameters in a study on meadow clover. It was observed that the application of vitamin B12 and humic acid delayed the flowering time by approximately 15 days, increased the total number of stems, and resulted in a 30% decrease in leaf trichome density and a 60% increase in root dry weight. In a study conducted by Torres-García et al. (2018) on the foliar application of a biostimulant based on cattle manure vermicompost (VCLB) leachate, including its effect on corn, cotton, and peanut yield, according to the results obtained from VCLB effect on maize plants, igholgholat was reported that chlorophyll content and crop yield increased by 12%, 15% and 10%, respectively, compared to chemical fertilization.

Inorganic Compounds

Inorganic compounds derived from organic substances can also be used in sustainable agriculture. Inorganic compounds formed by water, minerals, acids, bases, and salts help with the growth and development of plants. These components typically lack carbon, are inorganic, and are not produced within living organisms but are taken from the external environment in a preformed state. They have a structure that allows them to enter cells directly without being digested, and they primarily serve a regulatory function in living organisms (Anonymous, 2024b). Dactylis glomerata L. and Festulolium braunii by Godlewska and Ciepiela in 2013, the effects of different nitrogen doses and the application of a biostimulant containing auxin, gibberellin, cytokinin, polyamine and phytolamine on the actual protein and simple sugar contents were investigated and the biostimulant application increased the protein content by 15% and simple sugar content by 10% in Dactylis glomerata, while the protein content increased by 12% and simple sugar content by 8% in Festulolium braunii. As a result of the research, the average carbohydrate/protein ratio was found to be 1.07 and this ratio was among the optimal values for ruminants. Senthilraja et al. (2013) conducted both pot culture and field experiments on maize (Zea mays) plants to evaluate the effects of brewery wastewater on plant growth and physiological changes and the results showed that plants irrigated with 100% brewery wastewater had a 30% increase in biomass and 25% increase in chlorophyll content compared to the control group. In a 2018 study on alfalfa plants, Tytanite, a titanium-based biostimulant, was tested in combination with nitrogen fertilization and a 10% increase in chlorophyll content and a 9% increase in protein content was observed regardless of nitrogen fertilization (Godlewska and Ciepiela, 2018). In a study conducted by Ağırağaç and Çelebi in 2021, the effects of urban wastewater on heavy metal and nutrient content of Caramba (Lolium multiflorum cv. Caramba) plants were investigated. The results showed that 100% wastewater irrigation increased lead (Pb) content by 150% and cadmium (Cd) content by 120% compared to the control. Furthermore, nitrogen (N) and phosphorus (P) contents increased by 30% and 20% with 50% and 25% effluent treatments, respectively. However, higher effluent concentrations negatively affected plant health and growth rates.

Beneficial Fungi and Bacteria (Inoculants)

The biostimulant group includes especially biological fertilizers. These fertilizers include plant growth-supporting rhizobacteria (PGPR), some fungi, and mycorrhizae, which contain live microorganisms and can be applied to seeds, different surfaces of plants, and soil. When fertilizers in this group are used, an increase is observed in the nutrient uptake in plants, root area, and biomass, as well as the capacity of plants to remove nutrient elements from the soil (Vessey, 2003). These microorganisms are isolated from plant and soil residues, water, and composted organic fertilizers. On the other hand, PGPR (plant growth-promoting rhizobacteria) and PGPB (plant growth-promoting bacteria), among biological fertilizers, have been isolated from the rhizosphere region around the roots of plants. The key factor in the development of microbial inoculants is their commercial formulations. Selectively inoculated microorganisms should maintain their viability in commercial formulations and show the expected effect in the fields where they are inoculated. Similarly, it is also essential that these preparations applied from seeds or leaves are compatible with chemical fertilizers and plant protection products (Külahtaş and Çokuysal, 2016). Beneficial microorganisms, known as PGPRs, act as biostimulants. They perform tasks such as nitrogen fixation, making plant nutrients available, producing siderophores, facilitating iron uptake, and generating volatile organic compounds, and the genera to which these bacteria belong are mostly *Acetobacter, Acinetobacter, Achromobacter, Aereobacter, Agrobacterium, Alcaligenes, Artrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Clostridium, Enterobacter, Erwinia, Flavobacterium, Klebsiella, Microccocus, Pseudomonas, Rhizobium, Serratia* and *Xanthomonas* (Çakmaçı, 2005). *Azotobacter* bacteria have an important place as they increase the nitrogen cycle by 25-30% compared to normal nitrogen cycle with their different metabolic functions (Sahoo et al., 2013). It is known that these bacteria synthesize vitamins such as thiamine and riboflavin and hormones such as auxin, gibberellin, and cytokinin in addition to nitrogen fixation (Abd El-Fattah et al., 2013). It was reported that *Azotobacter chroococcum* microorganisms around the plant roots increased seed germination by 35% and promoted 40% and 30% improvement in root length and mass, respectively, compared to the control group (Gholami et al., 2009). *Azosprillum* spp. bacteria interact with plant roots and tissues inside the roots. In some studies, the effects of *Azosprillum* species on nitrogen content in plants were investigated. It was determined that 7-12% of the total nitrogen was formed in wheat by the activity of *Azosprillum brasilense* and *Azosprillum lipoferum* (Malik et al., 2002), and 60-80% of the total nitrogen content in sugar cane was formed by nitrogen fixation by *Azosprillum diazotrophicus* (Boddey et al., 1991). These fertilizers, which are especially recommended for plants such as corn, millet, and sorghum, can fix 2-4 kg nitrogen per decare per year, and they also produce plant growth regulators as a result of their metabolic activities (Okur and Ortaş, 2012). On the other hand, this bacterial species does not form nodules in plant roots. It has been known for many years that *Rhizobium* bacteria, due to their symbiotic life with plants, convert nitrogen in the atmosphere into usable nitrogen forms for plants, thus increasing the yield of cultivated plants (Sharma et al., 2011). These bacteria, resistant to different temperatures, generally enter the root from the root hairs, multiply, and form nodules in the root (Nehra et al., 2007). PGPR colonization of plant roots expands root architecture and improves nutrient and water uptake, nitrogen fixation, phytohormone production, enzyme production, photosynthetic activity, and other processes (Chieb and Gachomo, 2023). Some microorganisms increase the uptake of nutrients in the soil in the growth medium, thus allowing plants to take these elements more efficiently. It has been determined because of some studies that these bacteria convert phosphorus in the soil into available forms, and it is reported that microorganisms convert phosphorus forms that plants cannot take up into available phosphorus forms by producing organic acids (Kpomblekou and Tabatabai, 1994). Similar to phosphorus, potassium in the soil is also converted into available potassium, especially by Bacillus bacteria. The mentioned bacteria break down mica, illite, and orthoclase clay minerals in the soil with the help of the organic acids they produce, which release potassium ions (Sheng and He, 2006). Using bacteria that support plant growth in agriculture positively affects plants' nutritional status and protects plants against stress. While Paul and Nair (2008) reported that osmolytes and salt stress-induced proteins produced by Pseudomonas fluorescens bacteria and plants were 35% less affected by salt stress than control treatments, Baharlouei et al. (2011) reported that some Pseudomonas bacterial strains produced IAA, siderophore and ACC deaminase enzymes and protected canola and barley plants from heavy metal stress by promoting 20-40% more root and shoot growth. Sever Mutlu et al. (2019) and De Luca et al. (2020) stated that bacteria-based biostimulant applications positively affect grass quality, color, and density and meet the nutritional needs for post-cutting development in their studies on turf plants. In two separate studies, Sezen and Küçük (2021, 2023) investigated the effects of Microcystis viridis and Aphanizomenon gracile cyanobacteria on plant growth in areas cultivated with vetch (Vicia sativa L.), chickpea (Cicer arietinum L.), barley (Hordeum vulgare L.), maize (Zea mays L.), and lentil (Lens culinaris Medik). The studies demonstrated that cyanobacteria applications increased root length by 15-25%, plant height and root dry and fresh weights by approximately 30%, and green parts weight by 20% compared to untreated plants. These findings highlight the significant positive effects of cyanobacteria applications on plant growth and development relative to control groups. Dağ et al. (2024) conducted a study to determine the effects of microbial fertilizer containing *Azotobacter chrococcum* and *Azotobacter vinelandii* bacteria on yield and some yield components of two different corn varieties, and it was observed that it had significant effects on plant height by 8-11%, the first cob height by 21-24%, the cob length by 5%, the cob diameter by 1-3%, and the grain yield by 9-11% increase.

Mycorrhizae are fungal species that establish a symbiotic relationship with the roots of some plant species, allowing plants to take more nutrients from the soil with the help of mycelia and hyphae, and that play a supportive role rather than being parasitic on the plant. It has been determined that with the help of these effects of mycorrhizae, growth, development, and protection from pathogens and environmental stress factors are encouraged in plants (Lamabam et al., 2011). Studies have shown that these fungi take phosphorus, zinc, and other micronutrients that plants cannot take in the soil through their hyphae and carry them to the cortex cells in the root with the help of their mycelia (Smith et al., 2011). More than 96% of natural plant species are symbiotic with mycorrhizal fungi (Ortaş et al., 1999). The joint application of mycorrhizal fungi with beneficial bacteria positively affects plant growth by 20-40%, yield by 15-30%, nutrient uptake (N, P, K) by 10-25%, and environmental stress tolerance (drought, salinity, etc.) by 15-35% compared to control groups (Bhardwaj et al., 2014). In conditions where soil tillage is not done or is done at a minimum level, *Azotobacter, Azospirillum, Rhizobium*, and *Cyanobacteria* group bacteria in the soil make phosphorus and potassium available, while mycorrhizae increase the uptake of these elements (Doğan et al., 2011; Aziz et al., 2012; Oddi et al.,2024) A study was conducted on an artificial pasture with a mixture of 5 different forage plants using the Mycorrhizae microbial inoculum Micosat F and a chito-oligosaccharide mixture for the nutrient medium, and the seeds were inoculated with this mixture during planting and increased root colonization by 40%, plant species diversity by 25%, productivity by 30%, and weed control efficacy by 20%. In another study, Hai-Yang et al. (2024) concluded that single or combined inoculation of *Paraglomus occultum* and *Rhizobium leguminosarum* bv. *trifolii* significantly enhances nitrogen levels in both plant tissues and soil, with observed increases of up to 55% in plant nitrogen content and improved availability of ammonium and nitrate nitrogen in the soil.

Market Development and Use of Challenges

The biostimulant market has experienced substantial growth due to the global shift towards sustainable agriculture. Producers are increasingly considering the benefits of integrating biostimulants with conventional fertilizers due to oppressive environmental regulations, and ongoing research and development are yielding innovative formulations that will increase biostimulant effectiveness and meet the growing demand for environmentally friendly solutions. The global biostimulant market was valued at USD 3.5 billion in 2022 and is estimated to reach approximately USD 10.25 billion by 2032, with a growth rate of 11.40% from 2023-2032. The European market accounted for the highest revenue share at 38% in 2022, with a market share of USD 1.47 billion in 2023. It is expected to reach USD 3.86 billion by 2032, with a growth rate of 11.30% during 2023-2032 (Anonymous, 2024c). Although the use of pesticides and fertilizers is inevitable as long as agriculture is carried out, the target of reducing chemical pesticides and inorganic fertilizers by 50% by 2030 and developing environmentally friendly products instead has been set within the scope of adaptation to climate change and the European Union Green Deal (Maçin, 2021).

The agricultural use of biostimulants will provide solutions locally and temporally. Longer-term ecological effects should also be assessed and integrated into production. To achieve the benefits biostimulants can provide for profitable and sustainable plant production, stakeholders, farmers, public research, and regulatory institutions will be required to participate. The most critical points in using biostimulants are their application according to soil and plant type. Therefore, detailed research on biostimulants should be carried out effectively, and agricultural applications should be carried out first. Since the definition of biostimulants varies worldwide and legal regulations show profound differences between countries, the amounts of biostimulants used and the areas, where they are used should be precisely determined and added to the relevant legislation. In this long journey, public action is expected to harmonize policies and regulations and establish a robust risk assessment framework that respects the principle of proportionality and prevents duplication of data requirements between rules.

Conclusion

Agricultural production has become increasingly difficult due to stress factors such as drought, high temperature, and salinity, which have increased in recent years due to the effects of climate change. The fertility and structure of soils are deteriorating daily due to drought, salinity, high temperature, environmental pollution, excessive and unconscious chemical use, metal toxicity, and similar reasons, and it is becoming more difficult to obtain quality plant products. Despite this, the world population is increasing, and meeting the nutritional needs of this population is becoming more complex every day. Integrating biostimulants into agricultural production practices benefits plant growth and stress tolerance and contributes to the agricultural ecosystem's overall health. Promoting symbiotic relationships between plants, soil microorganisms, and the surrounding environment promotes soil fertility, improves nutrient cycling, and reduces the adverse effects of agricultural practices on soil health. Understanding biostimulants' mechanisms of action, their interactions with environmental stresses and plant genotypes, and their application in agricultural production is complicated and vital. It is crucial to develop tools to monitor the effectiveness of biostimulants and create management plans to optimize their use. It can be thought that biostimulants can be very useful for a sustainable life by revealing their full potential and having positive effects on plants, the environment, and human health.

Funding Information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

The authors declare no conflict of interest.

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