

Potential Impact of Green Biostimulants to Enhance Soil Health, Crop Physiology, Food Quality, and Agricultural Productivity Amidst Rising Global Population Demands

Kamal Prasad*

Privi Life Science, Research and Development, A-71, TTC Industrial Area, Thane-Belapur Road, Kopar Khairane, Navi Mumbai, Maharashtra, India.

**Corresponding author: Kamal Prasad.*

Abstract

The agricultural sector requires innovative strategies to enhance productivity and optimize resource use in response to the growing global population and the ongoing need for sustainable intensification of green agricultural production. Biostimulants have emerged as promising, environmentally friendly solutions that support sustainability across the entire production system. Compounds such as humic and fulvic acids, protein hydrolysates, algal extracts, chitosan and other biopolymers, inorganic substances, and beneficial fungi and bacteria have been shown to promote plant growth, increase crop yield, and improve product quality. Among these, microbial biostimulants-particularly beneficial fungi-demonstrate significant potential for enhancing plant performance. Some biostimulants also enhance nutrient uptake and improve plant tolerance to abiotic stress. A plant biostimulant is defined as any substance or microorganism that, when applied to plants, enhances nutrient efficiency, stress tolerance, and/or crop quality traits, regardless of its nutrient content. Commercial green biostimulants often contain blends of such substances and microorganisms. This definition is supported by scientific evidence on the nature, mechanisms of action, and effects of biostimulants on both agricultural and horticultural crops. Many biostimulants enhance nutrient efficiency independently of their nutrient content. Biofertilizers, considered a subset of biostimulants, improve nutrient use efficiency and open new pathways for nutrient acquisition in plants. These microbial biostimulants include mycorrhizal and non-mycorrhizal fungi, bacterial endophytes, and Plant Growth-Promoting Rhizobacteria (PGPR), all of which contribute to enhanced plant growth, development, and productivity. This manuscript focuses on the latest research and findings regarding the use of biostimulants in crops, emphasizing their role in improving quality and yield to meet the demands of a rapidly growing global population.

Keywords: PGPR; nutrient efficiency; green biostimulants

Introduction

The growing pressure on crop productivity (CP), including agriculture crops and fruits, caused by the increasing world population, is a demanding challenge, as the reduction of the use of agrochemicals with negative impacts on humans and ecosystems is compulsory. Hence, new strategies must be found, including those from the bio-based industry, using circular economy principles (Xu and Geelen, 2018; Verma et al., 2020). Crop production, yield, and quality are affected by numerous factors, including both biotic and abiotic stresses. Stresses caused by such factors can reduce production yield, sometimes significantly, but also negatively influence the quality characteristics of fruit (Di Vittori et al., 2018). The ability to regulate plant growth (PG) and, at the same time, to reduce the impact of these biotic and abiotic stresses, can provide tools to achieve maximum productivity and quality yield. Even though abiotic

stresses can be prevented to some extent by providing plants with their optimal growth conditions (including fertilization and watering) and using plant growth regulators (PGRs) (auxins, cytokinin's, gibberellins, strigolactones, brassinosteroids), the use of biostimulants is becoming a common practice in production systems (Yakhin et al., 2017; Roupael and Colla, 2020). Biostimulants have recently received significant interest in science and business due to their ability to alter physiological processes in plants related to growth, production, fruiting, and stress reduction. (Colla and Roupael, 2015; Basile et al., 2020, Prasad, 2022a). Furthermore, being bio-edible products, their impact on biodiversity, the environment, human health, and the economy is considerably less when compared to inorganic and organo-mineral fertilizers (Souri et al., 2019). Biostimulants exert their function by regulation of several physiological and molecular mechanisms,

including stimulation of the carbon (C) and nitrogen (N) metabolism, enhancing of antioxidant defence and production of metabolites, expanding photosynthetic action, and refining water relations, augmenting soil characteristics, both chemical and physical, enacting hormone-like exercises (especially particularly auxins, cytokinins, and gibberellins), improving epiphytic and rhizosphere microbial populations and modulating root system, regarding biomass, branching, density, diameter, length, and volume of soil/substrate exploited (Basile et al., 2020). Nevertheless, the beneficial results of using a particular biostimulant in one culture cannot be transferred to other types of crops, especially fruits grown in the open field than to fruits grown in greenhouse conditions. This difference is probably linked to the effects of application frequency and favourable climatic conditions in controlled environments (Colla and Roupheal, 2017; Petropoulos, 2020). To our knowledge, this is the first review aimed at providing an up-to-date overview of the known and potential benefits of plant biostimulants on crop growth, yield potential, and nutritional and functional quality. Biostimulants recognized benefits for human health that influence consumer acceptability (Blando and Oomah, 2019; Basile et al., 2021). Climate conditions can have a significant impact on agronomic traits such as fruit set, blooming, and fertilization. These traits can be troublesome since they can delay or cause irregular flowering, reduce bud opening, and impede pollination (Hussein et al., 2020) which can significantly influence the obtained yield. Other issues with fruit are linked to physiological disorders of fruits, namely cracking, pitting, wrinkling, and darkening of stalk colour, which can negatively influence the market price of this crop (Kantaroglu and Demirba, 2020). The present manuscript focuses on the most recent findings on the use of biostimulants in agricultural crops for quality

production and productivity for the extremely upcoming global population.

Biostimulants

A biostimulant is a substance or microbes that increases plant quality by stimulating natural processes in plants. A plant biostimulant is well-defined as components, except for nutrients and pesticides, which, when subjected to plants, seeds, or growing substrates in precise formulations can modify the physiological actions of plants in such a way that caters to prospective edge to growth, development, and/or stress response (Calvo et al., 2014; Du Jardin, 1915). There are many different types of biostimulants, including peptides, amino acids, polysaccharides, humic acids, and plant hormones. The constituents of the biostimulants are mineral elements, humic substances (HSs), vitamins, amino acids (AA), chitin, chitosan, and poly- and oligosaccharides. The meaning of plant biostimulants (PBs) has been strictly deliberated over the preceding few years, and lately underneath the new regulation. The aim is to improve the nutritional course of the plant, regardless of the nutrient content of the processed product, with the sole aim of improving one or more of the resulting properties of the plant or the plant rhizosphere such as the effectiveness of nutrient utilization, abiotic tolerance stress, quality characteristics, and/ or limited nutrient availability in the soil or rhizosphere. Based on this classification, PBs are defined according to their agricultural uses and include a variety of naturally occurring bioactive components, such as fulvic and humic acids, hydrolyzed animal and vegetable proteins, silicon, macroalgae, and seaweed extracts, in addition to beneficial microbes, such as nitrogen-fixing bacteria strains from the genera *Rhizobium*, *Azotobacter*, *Azospirillum* and arbuscular mycorrhizal fungi (AM fungi).

Table 1: Effects of biostimulants on Agriculture crops under field condition.

Types of Biostimulant	Technique of Application	Impact on crops
Humic acids	Soil application	Positive impacts on leaf and fruit chemical parameters, Increased leaf zinc and iron concentrations
	Soil application and foliar fertilization	Decreased leaf membrane degradation and increased peroxidase activity
	Foliar spray	Increased various mineral content of leaves, Leaf recovery from iron chlorosis
Fulvic acids	Soil application	Increased root and fruit dry matter and yield
Glycine betaine	Foliar spray	Fruit increased in size and content in soluble solids, polyphenols, vitamin C, and antioxidant activity, decreased stomatal conductance (gs) and increased relative water content, Increased fruit weight, and colour at ripening, Increased anthocyanin content, but decreased carotenoid and ascorbic acid content, Reduced fruit cracking incidence and increased wax content and cuticle and epidermal thickness
Seaweeds	Foliar spray	Higher production, yields, and improved status of leaves, Increased yield and fruit firmness Increased fruit weight, Reduced fruit cracking, Increased fruit set, and yield, Reduced fruit cracking and increased fruit dimension, Increased fruit size, soluble solids, polyphenols, and vitamin C content
Amino acids	Foliar spray & Soil	Progressive and uniformed sprouting and flowering
Chitosan	Foliar spray	Reduced storage decay
Silicate	Soil application	Improved fruit flesh firmness
Fungal-based biostimulants	Soil application	Enlarged root diameter, volume, fresh and dry weight
	Root inoculation	Enlarged shoot growth and plant development of rootstocks, Increased Zn and P uptake in rootstocks, Improved survival rate of rootstocks,
Bacteria based biostimulants	Soil application	Decreased effects of salt stress in saplings, Improved net photosynthetic rate, water use efficiency, and root vitality
Bacteria based biostimulants	Foliar spray	Improved yield, fruit weight, and shoot length, better leaf mineral content, increased fruit length, diameter, seed weight, and soluble solids content, Increased N, P, K, Fe, Zn, and Mn leaf content of seedlings

Principal Categories of Plant Biostimulants

Despite recent efforts to clarify the regulatory status of biostimulants, there is no legal or regulatory definition of plant biostimulants anywhere in the world, including in Asia, the European Union and in the United States. This situation makes a detailed list and classification of substances and microorganisms covered by this term impossible. Nevertheless, several major categories are widely recognized by scientists, regulators, and stakeholders (Calvo et al., 2014; Halpern et al., 2015) and include both microorganisms. Microorganisms include beneficial bacteria, primarily PGPR, and beneficial fungi. They may be free-living, rhizosphere, or endosymbiotic.

Definition and Principal Categories of Biostimulant

A precise definition of "biostimulants" has not yet been established, even though the body of research on the topic has expanded rapidly in recent years.

"Biostimulant" refers to products that fall into any of the following categories: metabolic enhancers, plant strengtheners, positive plant growth regulators, elicitors, allelopathic preparation, plant conditioners, phyto stimulators, biofertilizers, or biofertilizer/biostimulant (Yakhin et al., 2017, Prasad, 2021a; Prasad, 2022a, b). Definitions for what can be ultimately considered biostimulants have been evolving since the 1950s, starting from every living tissue (human, animal, and plant), when exposed to unfavourable but non-lethal situations undergo biochemical remodelling that results in the development of unique compounds that are non-specific biogenic stimulators that stimulate the organism's life reactions in which they are introduced. Plant biostimulants are substances whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to independently improve nutrient uptake, nutrient efficiency, abiotic stress tolerance and/or crop quality. (Ricci et al., 2019,

Prasad, 2021b). Plant biostimulants are natural resource-based microorganisms or substances in the form that are applied to plants, seeds, soil, and other substrates and made available to users for the benefit of activating and stimulating the natural processes of plants. Regardless of the content of nutrients or any combination of such substances and/or microorganisms intended for this purpose, so-called biostimulants, the efficiency of nutrient utilization and/or stress resistance. Their classification may be performed using the mode of action and the origin of the active ingredient or based on their action in the plants or on the physiological plant responses. Another suggestion has been put forward by Du Jardin (2015), stating that any definition of biostimulants should focus on the agricultural functions of biostimulants, not on the nature of their constituents nor their modes of action. As the classification of biostimulants has progressed, the classification of these products has also changed over the years. Subsequently, Ikrina and Kolbin (2004) proposed nine categories based on the raw materials utilized to create biostimulants. As of right now, du Jardin (2015) and Yakhin et al. (2017) have established the most frequently acknowledged taxonomy, which has seven categories. These sorts include humic and fulvic acids, protein hydrolysates and other nitrogen-containing compounds, algal extracts and botanicals, chitosan and other biopolymers, inorganic compounds, beneficial bacteria and beneficial fungi.

Properties of Biostimulants

The grouping of the biostimulant is not limiting, it can be a substance or a microbe. A substance is not always a fully classified structure; rather, it can be a unique chemical compound or a group of components having a clear biological origin, such as plant extracts. Biostimulants are demarcated by envisioned agricultural productivities. Nutrition efficiency might include nutrient utilization and absorption from the soil, root growth, transportation, accumulation, and absorption of nutrients in the plant. 'Abiotic stress' denotes to any physical or chemical stressor of a non-biological source. Different quality attributes may have different meanings, such as nutritional value, shelf life, or flower colour. Because they do not contain the nutrients meant to be transferred to the plant, biostimulants are not considered fertilizers. The target of these formulations is not to nourish with nutrients but to support and stimulate the metabolism of the plant, increase

growth, and productivity and reduce plant stress. Through a series of distinct processes, including the expansion of soil enzyme or plant growth hormone activities and the stimulation of soil microbial activity, they increase crop growth and yield (Piccolo et al., 2019). The main categories of plant biostimulants are elucidated in Table 1. Some major categories are universally acknowledged by scientists, regulators, and stakeholders (Calvo et al., 2014; Halpern et al., 2015) covering both substances as well as microorganisms (beneficial bacteria mainly PGPRs, and beneficial fungi). They can be free-living, rhizospheric, or endosymbiotic in nature.

Groups of Biostimulant

- a. *Organic Acids*: Typically include humic and fulvic acids.
- b. *Protein Hydrolysates and Other Nitrogen-Containing Compounds*: These are derived from enzymatic hydrolysis of proteins.
- c. *Amino Acid-Based Biostimulants*: Obtained from animal or plant sources; the most effective are typically extracted from plant matter, such as soybeans, using enzymatic processes.
- d. *Seaweed Extracts and Botanicals*: Derived from kelp, algae, or various terrestrial plants.
- e. *Chitosan and Other Biopolymers*: Often sourced from crustacean shells and used for their biostimulant properties.
- f. *Inorganic Compounds*: Include beneficial minerals such as cobalt and silicon.
- g. *Microbial Inoculants*: Contain beneficial microorganisms, including fungi and bacteria, that support plant health and growth.

Category of Biostimulants

Humic and Fulvic Acids as Biostimulants

Humic substances (HSs) are the innate components of the soil organic matter (SOM) which is not only the consequence of the degradation of plant, animal, and residue of microorganisms but also the metabolic products of soil microorganisms utilizing such degraded components. HSs are the assembly of some diverse substances which are initially grouped based on their atomic mass and whether they are soluble in humins, humic acids and fulvic acids. They are the ultimate yield of microbial breakdowns and chemical deterioration of dead flora and fauna in soils (Asli and Neumann Peter, 2010) and are believed to be the major organic molecules that normally occur in the universe (Thao and Yamakawa, 2009). It is reported as one of the most important constituents of soil

organic matter (Pilon-Smits et al., 2009). HSs have been acknowledged as indispensable contributors to soil fertility (SF), acting on the physical, physicochemical, chemical, and biological properties of the soil. In soil, HSs have appeared to perform a major role in numerous soil and plant activities like regulating the accessibility of nutrients, diffusion of oxygen and carbon between the troposphere and the soil and lethal chemicals alteration and transport (Pilon-Smits et al., 2013). Furthermore, HSs in soils influence the functioning of plants as well as the concentration and activities of microorganisms in the rhizosphere zone (Gomez-merino and Trejo-Tellez, 2015). Humic Acids (HAs) and fulvic acids (FAs) are part of the humic compounds in the soil, with the addition of humins (du Jardin, 2015) and derive from soil organic matter decomposed by plants, animals, and microorganisms, as well as from the activity of soil fauna (Czyz and Reszkowska, 2007; du Jardin, 2019). They are separated into three categories, based on their behaviour in inorganic acidic and alkaline solvents. HSs acids are soluble in basic media, FAs are soluble in both alkali and acid media, and humins are not extractable from soil (Berbara and Garcia, 2014). Their role in plant and soil functions has been evaluated by Berbara and Garcia (2014) and includes the control of nutrient availability, soil/atmosphere carbon and oxygen exchange, and the transformation and transport of toxic chemicals. Furthermore, plant physiology and the composition and function of rhizosphere microorganisms are affected by humic substances in soils (Vranova et al., 2011). HSs have been recognized for long as essential contributors to soil fertility, acting on the physical, physicochemical, chemical, and biological properties of the soil. Most biostimulant effects of HSs refer to the amelioration of root nutrition, via different mechanisms. One of them is the increased cation exchange capacity of soils containing polyanionic HS and the increased intake of macro and micronutrients due to the increased availability of phosphorus by HS, which leads to the precipitation of calcium phosphate. An alternative prominent contribution of HS to root nutrition is the stimulation of H⁺-ATPase within the plasma membrane, which advantages the free energy released by ATP hydrolysis to be exploited for the significance of nitrate and extra nutrients and converts into transmembrane electrochemical potential. Besides nutrients, proton pumping by plasma membrane ATPases also contributes to cell wall loosening, cell enlargement, and organ growth (Khan et al., 2009).

HSs seem to enhance respiration and invertase activities by providing C substrates. Hormonal effects are also defined, but whether contain functional groups recognized by the reception/signalling complexes of plant hormonal pathways, liberate entrapped hormonal compounds, or stimulate hormone-producing microorganisms is often unclear (du Jardin, 2015). The proposed bio-stimulation activity of HS also refers to stress protection. Phenylpropanoid metabolism is significant to the production of phenolic compounds, which are involved in secondary metabolism and various stress responses. The high molecular mass of HS has been shown to enhance the activity of key enzymes of this metabolism in hydroponically grown maize seedlings, suggesting stress response modulation by HS (Olivares et al., 2015; Schiavon et al., 2010).

FAs can be observed as the organic portion of soil, which can dissolve in alkaline as well as acidic medium (Schiavon et al., 2010). FA has higher total acidity, higher carboxyl groups, and more adsorption and cation exchange capacities as compared to HA (Bocanegra et al., 2006). They are the main factor involved in the chelation and movement of metal ions, like Fe and Al (Esteves da Silva, 1998). The specific roles of HAs on plants are plant growth, yield, nutrient uptake, amelioration of abiotic stress (salinity stress, moisture stress etc.), plant physiology and metabolism.

Protein Hydrolysates and Other N Containing Compounds

Amino acids (AAs) and peptide mixtures are obtained by chemical and enzymatic protein hydrolysis from agro-industrial byproducts, from both plant sources (crop residues) and animal wastes (collagen, epithelial tissues) (Halpern et al., 2015). Synthesis of chemicals can also be employed for individual compounds or mixtures of compounds. Other nitrogen-containing molecules include betaines, polyamines, and "non-protein amino acids." Although diverse in higher plants, their physiological and ecological roles are poorly characterized (Vranova et al., 2011). Glycine betaine is a special case of AA derivatives with known antistress properties (Chen and Murata, 2011). Other categories of biostimulants are protein-based hydrolysates PHs (mixtures of peptides and amino acids of animal or plant origin), individual amino acids (glutamic acid, glutamine, proline), and glycine betaine (Calvo et al., 2014; Baltazar et al., 2021). The former is prepared by several approaches, namely enzymatic, chemical, or thermal hydrolysis from

animal and plant residues (du Jardin, 2019; Halpern et al., 2015). The latter include the known structural twenty amino acids that take part in protein synthesis, but also those non-protein amino acids found abundantly in some plants (Calvo et al., 2014; Vranova, et al., 2011). PHs' application is mainly foliar, although they are also applied to substrate and seeds (Rouphael and Colla, 2020). PHs have demonstrated, both in open field and in greenhouses, the ability to increase nutrient absorption, density, length, and number of lateral roots (Garcia-Martinez et al., 2010; Gluszek et al., 2020; Baltazar et al., 2021), as well as improved enzymatic activity, increased photosynthetic rate and stomatal conductance, plant growth, and productivity, especially under environmental stress conditions (Colla et al., 2017; Kałuzewicz et al., 2017; Luziatelli et al., 2019).

Botanicals Biostimulants (BBs)

Botanicals are substances obtained from plants that are used as food ingredients in medicines and cosmetics, as well as in plant protection products (Seiber et al., 2014). Compared to seaweed, much less is known about its biostimulatory effects, as so far attention has focused on its agrochemical properties. However, there appears to be an opportunity to use them as biostimulants (Ertani et al., 2013; Ziosi et al., 2013). Furthermore, plant interactions in ecosystems are known to be mediated by plant active compounds, referred to as allelochemicals, which are receiving increasing attention in the context of sustainable crop management. Although crop rotations, intercropping, cover crops and mulching are used in the first instance to exploit allelochemical interactions between plants (named allelopathy), further attention should be paid to these chemical interactions for the development of new biostimulants. Botanicals are defined as substances extracted from plants that are to be used in pharmaceutical or cosmetic products, but also as food ingredients or plant protection products (Seiber et al., 2014). Botanical extracts are employed as insecticides, even though there is still much to learn about their potential as biostimulants. Nevertheless, there is evidence to support their usage as biostimulants (du Jardin, 2015). Indeed, recent works show how plant-derived extracts help to improve stress resistance or plant growth and fruit setting (Ali et al., 2019).

Seaweeds Extracts (SEs)

The kingdom Thallophyta includes watery plants known as seaweeds or macroalgae. These organisms

are often considered as an under-utilized bioresource, while many species have been utilized as food, industrial gums, and in therapeutic and botanical applications for the various centuries. More than 33% of the global biostimulant industry is made up of seaweed extracts (SWEs), whose worth is predicted to rise in the years to come. The use of fresh seaweeds as a source of organic matter and as fertiliser is ancient in agriculture, but biostimulant effects have been recorded only recently. This prompts the commercial use of SWEs and of purified compounds, which include the polysaccharides laminarin, alginates and carrageenan's and their breakdown products. Other constituents contributing to plant growth promotion include micro and macronutrients, sterols, N-containing compounds like betaines, and hormones (Craigie, 2011, Khan et al., 2009; EL Boukhari et al., 2020). Some of these compounds are only found in algal sources, explaining the increasing interest of the scientific and industrial communities in these taxa. Most of the algal species belong to the phylum of brown algae with *Ascophyllum*, *Fucus*, and *Laminaria* as the main genera, but carrageenan's originate from red seaweeds, which correspond to a distinct phylogenetic line. Product names of more than 20 SWE products used as plant growth biostimulants have been listed by Khan et al. (2009). SWEs comprise major and minor nutrients, amino acids, vitamins, cytokinins, auxin and abscisic acid like growth promoting substances and have been informed to encourage the growth and yield of plants, improve tolerance to environmental stress (Zhang et al., 2003; Zagzog et al., 2017; Zhang and Schmidt, 2000), increase nutrient uptake from soil (Turan and Kose, 2004) and enhance antioxidant properties (Verkleij, 1992). There are many literatures available on seaweed-based inoculants used as biostimulants to enhance plant resistance against biotic and/or abiotic stress, plant growth promotion and their effects on root/microbe interactions, have established several roles in soil formation, quality/biofortification, and plant health. Research using model plants has demonstrated that these effects are consistent with physiological, biochemical, and molecular processes (Wajahatullah et al., 2009; Pamela et al., 1914). Any betterment in agricultural process that marks a better productivity must decrease the harmful environmental effect on agriculture and increase the self-sufficiency of the system. In a move to depict the possibilities of seaweed extract acting on rhizosphere nutrients availability, investigations were conducted

to describe their interaction with rhizosphere microorganisms and enzyme activity (Table 2). The applications of biostimulants can improve the efficiency of regular inorganic fertilizers. It has been reported that in agricultural and horticultural crops, marine bioactive compounds isolated from marine algae are used, which have various positive effects resulting in improved productivity and quality (Crouch and Van Staden, 1994; Bulgari et al., 2015). The advantageous effects of seaweeds (microalgae) and their extracts on crop systems have been used for innumerable period to upgrade the soil properties and to increase productivity and value of agriculture and horticultural crops. Liquid concentrate derived from seaweeds has lately earned significance for use as foliar applications for various plant species such as cereal, flowers and vegetables. Various poly and oligomers of naturally occurring or (semi) man-made compounds are progressively adopted in agriculture as inducers of plant defence, for example, seaweed polysaccharides. The option of seaweed utilization in contemporary agriculture has been extensively discovered and diverse varieties of formulations of these marine algae as liquid fertilizer and either whole or finely chopped powdered algal manures are utilized. The best effects of the use of SWEs were described for stimulation of seed germination and root growth, improvement of frost resistance, augmented nutrient uptake and control of phytopathogenic fungi, bacteria (Alves, 2016), insects or other pests (Asha, 2012) and for re-establishment of plant growth under high salinity stress (Nabti et al., 2010; Pacholczak et al., 2016). SWEs act on soils and on plants and can be applied on soils, in hydroponics solutions or as folia treatments (Craigie, 2011). In soils, their polysaccharides help in gel formation, water retention and soil aeration. The polyanionic compounds help in the fixation and exchange of cations, fixation of heavy metals and soil remediation. In addition to their other functions concerning hormone-related effects on seed germination, plant establishment, growth, and development, they also serve as fertilizers in plants, which is thought to be one of the primary causes of biostimulation activity in crop plants.

Chitosan and Other Biopolymers

Chitosan, a deacetylated product of the biopolymer chitin, is synthesized both naturally and artificially. Many foods, cosmetic, medical, and agricultural industries use polymers and oligomers in controlled and varied sizes. The physiological response of

chitosan oligomers in crops is not only the consequence of the abilities of this polycationic substance to associate with broad categories of cellular components, such as DNA, plasma membrane and cell wall constituents but owing to associate with unique receptors related to activation of gene involve defence mechanism, a way similar to plant defence elicitors (El Hadrami et al., 2010; Ávila et al., 2011; Hadwiger, 2013; Katiyar, 2015). Chitosans have been found extensively applied as a coating agent for a variety of fruits chiefly for protecting against post-harvest damages owing to pathogen infections (El-Sawy et al., 2010; Basit et al., 2020). The latest transcriptomic study highlighted that chitosans induce activities of genes responsible for varieties of events in plant life processes like SAR, plant defence system, photosynthesis, hormone metabolism, translation of HSP(s) and changing the metabolism of protein increasing content of storage proteins (Xoca-Orozco et al., 2017). Malerba and Cerana (2018) reported an augmentation of storage life and maintenance of anthocyanin content in strawberry fruit encrusted with chitosan. Rahman et al. (2018) study revealed that the appliance of chitosan on the strawberry's leaves notably improved the fruit growth and yield (up to 42% higher) than the control. The fruit from plants treated with chitosan show considerably higher contents of carotenoids, anthocyanins, flavonoids and phenolics than the control. The antioxidant expression in the fruit of chitosan-sprayed plants was extensively higher than control. Chitin and chitosan base products were used as unique receptors and signaling pathways. They are the major players in responding to stress stimulus as well as in the developmental regulations, Among the cellular consequences of the attaching of chitosan to approximately definite cell receptors, hydrogen peroxide aggregation and Ca^{2+} efflux into the cell were shown, seem to cause an enormous functional change. One possible explanation for the positive impact of chitosan (CHT) on crop response is its capacity to provide plants with more nutrients (Malerba and Cerana, 2018). In wheat cultivated in zinc-deficient conditions, spraying zinc and CHT complexes effectively supplies the minor nutrient (Deshpande et al., 2017). By combining CHT with waste silica, farmers may be able to apply less NKP fertilizer, which would have positive effects on the environment and their bottom line (Gumilar et al., 2017) Furthermore, CHT nanoparticles could be employed as a delivery system for plant growth

regulators. Such CHT nanoparticles complexed with GA notably enhance the leaf area and content of chlorophylls and carotenoids in *Phaseolus vulgaris* (Espirito et al., 2017). Thus CHT, which are obtainable in huge amounts and cheaply can be used in the development of sustainable agricultural systems and the production and preservation of food.

Inorganic Compounds

Chemical compounds that enhance plant growth and development, which could be necessary for certain categories of plants are known as beneficial elements (Basile et al., 2021). Major beneficial elements such as Al, Co, Na, Se and Si are generally found in soils and plants as inorganic compounds and in the graminaceous group as insoluble form (amorphous silica ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$). Such types of positive effects can be constitutive, like the hardening of cell walls through silica deposition, or exhibited in specific environmental circumstances, like pathogen attack for selenium or osmotic stress for sodium. The description of beneficial elements is not restricted to their chemical natures, but essentially to their beneficial impact on plant growth and stress response. There are numerous scientific studies on the impacts of beneficial components that improve plant growth, product quality, and resistance to a variety of abiotic challenges. These include cell wall rigidification, osmoregulation, lower rate of transpiration by crystal deposits, thermal manipulation through radiation reflection, enzyme activity by co-factors, plant nutrition via interacting with different elements during uptake and mobility, antioxidant protection, interactions with symbionts, disease-causing microbes and herbivore reaction, protection against the toxic effect of heavy metals, plant hormone productions and signaling (Pilon-Smits et al., 2009). Minerals salts of beneficial and essential elements (chlorides, phosphates, silicates, and carbonates) have been used as fungicides (Deliopoulos et al., 2010). Phosphite (reduced phosphate) is usually treated in soils in the form of phosphorus acid, phosphite salts with metal ions (K^+ , Na^+ , NH_4^+) and non-metallic anions (Deliopoulos et al., 2010). Certain facts indicate the biostimulant and fungicidal properties of Phi products, however, phytotoxicity risk might be associated if its rate is not maintained properly i.e., beyond 5 g/l or 36 kg/ha its effect is phytotoxic (Hardy et al., 2001; Barrett et al., 2003; Deliopoulos

et al., 2010). It can be applied to soil as a pesticide, supplemental fertilizer or biostimulant (Gomez-Merino and Trejo-Tellez, 2015) even though its effect as a fertilizer and growth enhancer is a matter of discussion. Various evidence indicated that Phi might behave as a biostimulant by regulating sugar metabolism and intercellular hormonal changes (Luziatelli et al., 2019), enhancing defence activities and shifting plant P nutrition (Varadarajan et al., 2002). Odoh (2017) reported that soil-saturated phosphite inhibits the establishment of the endoparasitic nematodes *Heterodera avenae* and *Meloidogyne marylandi* in both wheat and bristle oat plants. However, even though cereal crops appear to be responding to disease control, more research in the field is required to determine the extent and consistency of any likely beneficial impacts. Silicon is a beneficial element in some monocots, like rice, sugarcane etc. Its advantageous qualities include boosting resistance to infections and illnesses as well as endurance under abiotic stress. In the soil, either Si is presented as insoluble quartz or silicates that chemically bind with metals or as non-ionic silicic acid, which plant roots can take up effortlessly and translocated all over the plant system (Savvas and Ntatsi, 2015). Maximum concentrations are generally located at adjoining areas of the stomata. These silica deposits or phytoliths boost leaf mechanical vigour and stiffness, thus raising light absorption and photosynthesis. They also modulate nutrient and water mobility, apparently owing to silica gel form in the cell walls, which thus lessens the transpiration. Another stress-mitigating effect of Si may be attributed to its capability to halt toxic metal translocation in plant cells and the soil and to reduce the progress of plant senescence (Albrecht, 2019). As fungicides, inorganic salts of silicates, carbonates, phosphates, phosphites, and chlorides of important and beneficial elements have been employed (Deliopoulos et al., 2010). Although the modes of action are not yet fully established, these inorganic compounds influence osmotic, pH and redox homeostasis, hormone signaling, and enzymes involved in stress response (peroxidases). Their function as a biostimulant of plant growth, acting on nutrition efficiency and abiotic stress tolerance, hence distinct from their fungicidal action and their fertilizer function as sources of nutrients, deserves more attention.

Table 2: Positive effect of seaweed extracts on the available nutrients and Nutrient use efficacy to the whole soil and plant system.

Shoot Growth	Leaf Surface Area (+)
	Shoot Length (+)
	Number of Leaves (+)
	Number of Branches (+)
	Number of Fruit set (+)
	Yield (+)
Plant Fruit Quality	Hydrophilic antioxidant Activity (+)
	Vitamin C (+)
	Total Phenols (+)
	Soluble Solids (+)
	Fructose content (+)
	Sucrose Content (+)
Plant Physiology	Chlorophyll Content (+)
	Stomatal Conductance (+)
	Photosynthesis rate (+)
	Electron transfer rate (+)
Phytohormones	Auxin (+)
	Cytokinin (+)
	Gibberellin (+)
	Abscisic Acid (+)
Shoot Growth	Leaf Surface area (+)
	Shoot Length (+)
	Yield (+)
	Yield Quality (+)
Soil Activity	Hydrogenase Activity (+)
	Urease (+)
	Proteinase (+)
	Phosphatase (+)
	Invertase (+)
	Microbial Activity (+)
	Water Retention Capacity (+)
	Soil Texture (+)
Root Growth and Activity	Root Elongation (+)
	Root Nodulation (+)
	Root Architecture (+)
	Root Biomass (+)

Microbial Inoculants and Biofertilizers

A biofertilizer is a bacterial or fungal inoculant applied to plants to increase the availability of nutrients and their utilization by plants, regardless of the nutrient content of the inoculant itself. Biofertilizers may also be defined as microbial biostimulants improving plant nutrition efficiency and increase productivity.

Beneficial Fungi as Biostimulants

Fungi interact with plant roots in different ways, from mutualistic symbioses (when both organisms live in direct contact with each other and establish mutually

beneficial relationships) to parasitism (Adrian et al., 2014; Adnan et al., 2019, Prasad, 2022a). Plants and fungi have co-evolved since the origin of land plants. The concept of a symbiotic-parasitic continuum helps explain the expansion of relationships that have evolved throughout evolution. Mycorrhizal fungi are a heterogeneous group of taxa that establish symbioses with over 95% of all plant species. Among the different forms of physical interactions and taxa involved, the Arbuscule Mycorrhizae Fungi (AM fungi) is a widespread type of endomycorrhiza associated with crop and horticultural plants, whereby root cortical cells are penetrated by fungal hyphae of

Glomeromycota species to generate branched formations known as arbuscules (Clark and Zeto, 2000; Prasad, 2021b). To promote sustainable agriculture, the benefits of symbiosis for plant nutritional efficiency (both macronutrients, especially phosphorus and micronutrients), water balance, and protection from biotic and abiotic stresses are well recognized. There is growing interest in the use of mycorrhizae (Colla et al., 2008; Prasad, 2021a, b; Prasad 2022a, b; Prasad, 2023a, b). Recent discoveries indicate the existence of not only fungal and plant partners, but also hyphal networks that connect individual plants within plant communities. This could have significant ecological and agricultural implications since there is evidence that the fungal conduits allow for interplant signaling (Johnson and Gilbert, 2015; Simard et al., 2012, Prasad, 2022a). As a further area of research, AM fungi form tripartite associations with plants and rhizobacteria which are relevant in practical field situations (Siddiqui et al., 2008; Prasad, 2021a; Prasad, 2022b). To exploit the benefits of mycorrhizal association, plant management practices and plant varieties must be adapted to interact with microorganisms (Plenchette et al., 2005; Gianinazzi et al., 2010; Sheng et al., 2011, Prasad, 2022a, Prasad, 2023a). Metagenomics is an interesting tool to monitor and study microbial associations in the rhizosphere. Inoculation of plant propagules and soils complements these approaches (Sensoy et al., 2007; Sorensen et al., 2008; Candido et al., 2013; Candido et al., 2015; Colla et al., 2015; Sarkar et al., 2015). Biostimulants should include fungus-based treatments that are sprayed on plants to improve their nutritional efficiency, stress tolerance, crop output, and quality of final product. Major limitations on their use are the technical difficulty of propagating AM fungi on a large scale, due to their biotrophic character (Prasad 2021b; Prasad, 2023a) and, more fundamentally, the lack of understanding of the determinants of the host specificities and population dynamics of mycorrhizal communities in agroecosystems. Nevertheless, other fungal endophytes such as *Trichoderma* sp. (Ascomycota) and *Sebacinales* (Basidiomycota, including *Piriformospora indica* as a model organism), unlike mycorrhizal species, live at least part of their life cycle outside the plant and colonize roots and send the host using a poorly understood mechanism that can supply nutrients as recently shown (Uma et al., 2017). They are becoming more and more popular as model organisms for studying the mechanics of nutrition

transfer between fungal endosymbionts and their hosts, as well as easier-to-multiply in vitro plant inoculants. Some of these fungi, particularly the *Trichoderma* have been extensively investigated and studied for their bioinsecticide (fungal parasite) and biocontrol (induction of disease resistance) abilities and are used by the biotechnology industry as a source of enzymes. There is compelling evidence that many plant responses are also induced, including increased tolerance to abiotic stresses, improved nutrient utilization efficiency, and organ growth and morphogenesis (Shoresh et al., 2010; Colla et al., 2015; Hasanuzzaman et al., 2018). Based on these effects, these fungal endophytes may be regarded as biostimulants, though their agricultural uses are currently supported by claims as biopesticides.

Beneficial Bacteria as Biostimulants

Bacteria interact with plants in all possible ways (Ahmad et al., 2008; Prasad, 2021a, Prasad, 2022a): (i) As for fungi there is a continuum between mutualism and parasitism; (ii) bacterial niches extend from the soil to the interior of cells, with intermediate locations called the rhizosphere and the rhizoplane; (iii) associations can be temporary or permanent, and some bacteria can also be transmitted vertically via seeds (iv) functions that influence plant life include participation in biogeochemical cycles, provision of nutrients, improved nutrient utilization efficiency, induction of disease resistance, improved abiotic stress tolerance, and plant growth includes regulation of morphogenesis by regulatory factors. Within this taxonomic, functional, and ecological variety, two primary categories of biostimulants should be taken into account for agricultural applications: (i) *Rhizobium*-type mutualistic endosymbionts and (ii) mutualistic, rhizospheric PGPRs. *Rhizobia* and related taxa are traded as biofertilizers. As a microbial inoculant to enhance the uptake of nutrients by plants. GPRs are multifunctional and influence all aspects of plant life, including nutrition and growth, morphogenesis and development, responses to biotic and abiotic stresses, and interactions with other organisms in agroecosystems (Ahmad et al., 2008; Baltazar et al., 2021; Prasad, 2022a, b). *Rhizobia* and related taxa are commercialized as biofertilizers. As a microbial inoculant to improve nutrient uptake by plants. The biology and agricultural applications of *rhizobium*-based symbiosis are widely discussed in scientific literature and textbooks. Some of these functions are commonly performed by the same organism, some are strain-specific, and others rely on

synergy within the bacterial consortia. Agricultural applications of PGPRs are limited by this complexity and the different responses of plant varieties and recipient environments. Technical difficulties in formulating inoculum also led to inconsistent results in practice (Ali et al., 2019; Carillo et al., 2020). Nevertheless, the global market for bacterial biostimulants is growing, and PGPR inoculants are now considered a type of plant-based "probiotic" (Abdipour et al., 2020; Ashour et al., 2021; De Bellis et al., 2021).

Plant Growth Promoting Rhizobacteria (PGPRB) and Arbuscular Mycorrhizal Fungi

Microbial inoculants are mostly free-living bacteria, fungi, and AM fungi (Prasad, 2021a, b; Prasad, 2022a, b, c; Prasad, 2023a, b) which were screened from diverse situations such as soil, plants, plant residues, water, and composted manures. There has been evidence of improved absorption of basic elements including N, P, and K when PGPB is present. Certain PGPB species (*Azotobacter* and *Azospirillum*) can transform atmospheric nitrogen into a form that can be used by the plants (Calvo et al., 2014). Various evidence also highlighted the phenomenon where PGPB (s) enhance the solubility of certain essential elements, thereby improving nutrient accessibility for plants. This is mainly significant for elements like P which are generally not accessible to plants. Several microbes can also increase the solubility of potassium (K) from rock K minerals, by forming chelate with silicon ions, increasing its solubility and using organic acids to dissolve rock K. Therefore, application of PGPR at proper dose possibly will facilitate reduction in the rate fertiliser applications (N and P). Some PGPB may promote plant development through interactions with phyto-hormones. A variety of plant growth regulators like auxins, cytokinins, gibberellins and ethylene are identified to be synthesised by PGPB (Dodd et al., 2010), which can enhance both shoot and root growth of the plant (Lugtenberg et al., 2009). PGPB can also enhance the ability of plants to tolerate stress by disrupting certain stress hormones (ethylene) or altering the plant hormone interaction (Dodd et al., 2010). Some species of PGPR produce an enzyme that converts the originator for ethylene into 2- 2-oxybutanoate and NH₃. Thereby, decreasing both abiotic and biotic stresses like pathogenic bacteria, heavy metals, salt, and drought (Ahmad et al., 2008; Lugtenberg et al., 2009, Prasad, 2022a, b). Beneficial fungi having biostimulant activity such as symbiotic fungi, principally AM fungi in the genus *Glomus*,

(forms a well-branched network of roots and hyphae) enable the plants to widen their root arrangement further than the exhaustion region, thus allowing them to enhance the uptake of minerals and moisture improving their stress tolerance capacity. Other strains of non-pathogenic fungi having potential biostimulant activities that associate with roots include *Aspergillus*, *Trichoderma*, *Penicillium*, *Saccharomycetes*, *Mortierella* and *Mucor*. They have been established to enhance plant growth, increase plant nutrition, and improve tolerance to abiotic and biotic stress (Owen et al., 2015). *Trichoderma* having symbiotic relations with crops (promoting branching of roots) are found to secrete active solubilising agents to the rhizosphere and thus promote translocation of essential elements (Lopez Bucio et al., 2005; Layek et al., 2018) and capability to infect other fungi, they are frequently used as biocontrol agents for controlling fungal diseases of crops. Biocontrol microorganisms (BCMs) can be employed as activators of specific absorption rate (SAR). Simultaneously, fungal BCMs can enhance crop growth and development thus responding as PGPMs, sequentially govern an increased tolerance against abiotic stresses viz., drought, salinity etc. The capability of BCMs and PGPMs to regulate plant defence mechanisms, like SAR, was revealed, but the overall information of this BCM-plant molecular cross-talks is not understood properly, and many defensive substances may present but need to be recognized and isolated. There could be a lot of protective compounds present, but they must be identified and isolated. BCMs and PGPMs can be classified as "biostimulant microorganisms", which can promote crop growth and protect against pathogens throughout the plant life process i.e., seed germination to plant maturity. PGPR formulations are one of the important biostimulant classes due to their ability to improve root growth, mineral availability, and nutrient use efficiency in crop rhizosphere (Carvalho et al., 2014). Microbial soil balance has often been ignored by modern agriculture, however, biostimulation through soil microorganisms is now gaining momentum.

Plant Biostimulant

Any material regardless of nutritional content, that is administered to plants to improve crop quality attributes, abiotic stress tolerance, and/or nutrition efficiency is called a plant biostimulant. In a broader sense, herbal biostimulants also refer to commercial products containing mixtures of such substances and/or microorganisms.

The Nature of Biostimulants

Substances and microbes are involved for plant growth and development. Substances can be single compounds (glycine betaine) or groups of compounds of single natural origin of which the composition and bioactive components are not fully characterized (seaweed extracts); the substances commented by this review are naturally produced organic compounds, or inorganic molecules, but synthetic compounds should not be excluded, particularly if biostimulants contain specific plant growth regulators. Although nitrophenolates are synthetic phenolic chemicals that are registered as plant production goods, they are marketed and labeled as "biostimulants." Microbial inoculants may contain single strains (*Bacillus subtilis*) or mixtures of microbes showing additive or synergistic effects on plants growth and development.

Functions of Biostimulants

Physiological Functions of Biostimulant

Among the physiological processes are lateral root growth and safeguarding the photosynthetic apparatus from photodamage. Functions are supported by cellular mechanisms, like reactive oxygen scavenging by antioxidants or increased synthesis of auxin transporters. Physiological processes and the underlying cellular mechanisms are sometimes referred to as the biostimulants' "modes of activity." Finally, these modes of action explain the agricultural functions of biostimulants, e.g., increased tolerance to abiotic stress (causing oxidative stress), or increased N use efficiency (This depends on the food absorption capacity of the roots (the density of lateral roots). Agricultural activities have the potential to offer economic and environmental advantages, such as greater crop yield, reduced use of fertilizers, better crop product quality and profitability, improved ecosystem services, etc.

Agricultural Functions of Biostimulant

Biostimulants enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits. Quality characteristics may be related to nutritional value, grain protein content, shelf life, etc. These convergent effects should form the basis of any definition of a biostimulant. Stimulation of pathogen responses by triggers and plant gene regulators is also achieved by many of the described biostimulants, such as chitosan, laminarin, and some PGPRs. However, there is a growing consensus among regulators and stakeholders to separate biostimulation and biocontrol from a regulatory perspective. Therefore,

biotic stress is excluded from the scope of the definition.

Economic and Environmental Benefits of Biostimulant

Although these aspects provide incentives for the development of biostimulants, they should not form the basis for a scientifically sound definition of biostimulants. In conclusion, any definition of biostimulants should focus on the agricultural functions of biostimulants, not on the nature of their constituents nor their modes of actions, as they have been defined above.

Modes of Action of Biostimulants

The mechanism/course of action signifies a specific effect on a discrete biochemical or regulatory process. The course of action of all but a few biostimulants continues unnoticed. According to Bhupenchandra et al. (2020), this is chiefly owing to the diversified property of natural resources utilized for production and the composite nature of the constituent confined in biostimulant, a product that makes it nearly impossible to identify the precise factor or factors responsible for biological activity and to identify the relevant mechanism or mechanisms of action. Thus, importance must be given to the recognition of the "mechanisms of action" of biostimulants as shown by a natural specific outcome on plant yield through improvement in mechanisms such as photosynthesis, senescence, modulation of phytohormones, uptake of minerals and water, and regulation of genes involved for tolerance to environmental stresses and transformed crop canopy structure and phenology. A model of this development is the approaches that imply the use of protein-based biostimulants for which advanced studies have recognized the specific metabolic process and some of the systems over which they exercise their effect on crops. To strengthen our knowledge on the course of action of biostimulants the following steps of biostimulants activities, after their application on plants have been established: (1) diffusion into plant cells, translocation, and transformation in plants, (2) gene regulation, plant signaling and control of hormonal status, (3) metabolic activities and consolidated whole plant reaction.

Application of Biostimulants

Agronomic and Physiological Traits of Crops

The enhancement of the plant development process along with crop yield because of the application of PBs has been generally correlated to the activity of

signalling biologically active elements in the primary and secondary metabolisms (Rouphael and Colla, 2020). Diverse types of hydrolysed collagen, like granulated gelatin, gelatin hydrolysate and amino acid mixtures resembling gelatin composition were determined about plant growth in cucumbers (Yakhin et al., 2017). In their experiment, they revealed that the application of gelatin hydrolysate enhanced the activity of genes encoding for amino acid permeases (AAP3, AAP6) and transporters of amino acids and nitrogen. As a result, they concluded that gelatin hydrolysate acted as a biostimulant and a constant source of N. The application of these natural elements or microorganisms not only improves the reactions of microbial and non-microbial PBs but also enhances tolerance to environmental and biotic stresses.

Implications of Biostimulants for Abiotic Stresses Tolerance

Global climate change (GCC) such as adverse ecological conditions and edaphic factors viz. drought, salinity, and extreme weather, imposed about a 70% yield gap (Wang et al., 2003). Under substantial changes in the global climatic condition, these environmental stresses are likely to have a highly harmful impact, imparting serious effects on crop production, and hence global food safety (Rouphael et al., 2018). Thus, it has been suggested that using both microbial and non-microbial PBs is a crucial and effective way to stabilize crop yield. Additionally, microbial and non-microbial PBs might be considered a workable method to boost N's resistance to salinity and act as a biostimulant. These positive consequences may be correlated to various physio-chemical processes such as reduced membrane lipid peroxidation, enhanced chlorophyll content, better antioxidant activities and an improved afflux and compartmentalizing of the intracellular ions.

Improving Nutrient Use Efficiency of Biostimulants

The utilization of biologically active native elements and microbial extracts can signify an important means to augment nutrient status in the soil, plant nutrient absorption, translocation, transformation, and metabolism (De Pascale et al., 2017). Enhancing nutrient utilization efficiency in particular N and P is essential from both economic and environmental points of view. At both ideal and low N regimens (112 and 7mg L⁻¹, respectively) the use of legume-based PH specifically such as substrate drench increases the number of leaf Soil Plant Analysis Development

(SPAD) index and biomass production of greenhouse (Savvas and Ntatsi, 2015). The improved agronomic feedback of Ph-applied tomato was related to the improvements of root architecture that resulted in better N uptake and translocation. Additionally, in roots with unsatisfactory N levels, PH treatment increased the expression of genes encoding for glutamine synthetase, ferredoxin-glutamate synthases, and amino acid transporters, all of which are known to be essential for N metabolism.

Enhancing Quality Produce Through Biostimulants

The microbial and non-microbial plant biostimulant application can alter a plant's metabolism both primary and secondary (Colla et al., 2015, Prasad, 2021b) which results in the synthesis as well as the build-up of antioxidant compounds (secondary metabolites) that vital for human nutrition. This treatment also causes a considerable boost in tomato fruit quality such as antioxidant capacity, total soluble sugars, carotenoids (lycopene, lutein, and b-carotene), total polyphenols and flavonoids contents in addition to the mineral composition (P, K, Ca, Mg, Fe, Mn, and Zn). Furthermore, Haplern et al. (2015) experimented on two Brassica species: Brassica campestris and Brassica juncea and studied the effect of PAR (photosynthetically active radiation) (low or high). The effects of phosphate (high or low) and phosphite (low, optimal, or high) and their interactions with glucosinolate, flavonoid, and nitrate levels. As a potential defense mechanism against nutrient stress, they noted that adding phosphite to the nutrient medium may increase phosphate deficit and thereby promote the production and aggregation of many target flavonoids and glucosinolates.

Science and Practice of Biostimulants

The scientific community and commercial enterprises have an immense interest in the identification of bioactive components of PBs and explicating the stimulation mechanisms at molecular and physiological levels. The most proficient technology to develop novel biostimulants is the use of small/medium/large/high throughput phenotyping, because of complex matrices having diverse groups of bioactive and signalling molecules (Rouphael et al., 2018). The niche of biostimulants is a baffling concept (Torre et al., 2013) and it has made sure that the market for biostimulants is not founded on efficacy or science, and that many items are little more than recycled waste products with marketing and

pseudoscience behind them. On biostimulant products, several research studies have been conducted and it is futile or have inactive, unstable, or inconsistent properties and some have negative effects when compared with well-designed controls (Csizinszky et al., 1984; Di Marco and Osti., 2009; Cerdan et al., 2013; De Oliveira et al., 2013; Carvalho et al., 2014). It was reported that "none of the biostimulants tested achieved sufficient levels of pathogen control to justify replacement or supplementation with traditional synthetic fungicides" (Banks et al., 2012) and it poses positive and negative impacts and as recent biostimulants are heterogeneous mixtures obtained from natural resources of highly varied origin and manufacture using highly diverse techniques thus it can be expected to have a wide range of potential biological activity and security. Numerous experiments have suggested that linking numerous biostimulants could offer steady effects as compared with their treatment. Our understanding of one biostimulant or one plant species cannot be readily applied to another biostimulant or another plant species since stimulating effects are species- and product-specific. To expand both basic and applied science regarding the efficacy of biostimulants for a particular plant species, it is necessary to perform a broad spectrum of research on this species, with a variety of products, treatments, growth stages, etc. PBs such as natural substances and microbial inoculants seem like a novel and possible group of agricultural inputs, complementing agrochemicals including synthetic fertilizers, enhancing tolerance to abiotic stresses and enhancing the standard of horticultural and agricultural products concurrently.

Nature, Scientific and Regulatory Challenges of Biostimulants

The greatest scientific challenge is the complexity of the physiological effects of biostimulants. In general, the main action of biostimulants is to induce physiological responses in plants. Numerous of these reactions have an impact on growth, development, and basic metabolism. These processes are subject to strict homeostatic control derived from millions of years of biological evolution, allowing plants to occupy specific ecological niches and produce characteristic phenotypic responses to fluctuating environments. Influencing such biological processes is difficult and requires attention to the many interactions between processes and signaling pathways as plant organisms respond to their

environments, the tripartite interactions between biostimulants, plants, and the environment must be appropriately considered for the usage of biostimulants to be successful. For example, phosphorus mobilization using phosphatase-releasing PGPR may be difficult for plants when inorganic phosphate is limited in the soil or when plants contribute to the maintenance and activity of PGPR inoculum in the rhizosphere. Formulating and combining biostimulants with conventional fertilizers and/or crop protection chemicals present technical obstacles. Many biostimulants aim to improve nutrient utilization efficiency, and the combination of fertilizer and biostimulants needs to be optimized. Formulation of biofertilizers is especially complex, and positive interactions between microbiological components of the biostimulant mixtures on one hand, and between the biostimulant inoculant and the resident rhizo-/endospheric microbiota on the other hand. Technical difficulties also arise in the monitoring of crops and in deciding on whether, when and how biostimulants should be applied. The situation is more complex than with any plant protection products, for which the incidence of pests and diseases is relatively easy to detect and quantify, and for which epidemiological models are available to optimize pesticide applications. Such a situation with abiotic stressors, which often interact in the field, is difficult to assess and is usually quantified ex-post by yield penalties. Nutrient use efficiency is also difficult to evaluate and the decision to apply a biostimulant that would target this trait is hardly justified by measurable plant characteristics in the field. Particular challenges to intellectual property protection arise from biostimulants. Patenting biostimulation products and preventing copying/reverse engineering is often difficult. Originality of the product and its status of invention, as demanded by patentability, is sometimes difficult to establish, and companies tend to patent the industrial processes used to produce biostimulants. Data protection mechanisms coupled with the mandatory registration of biostimulants would strengthen the protection of intellectual property. Data sharing mechanisms related to the registration of biostimulants, or microorganisms support the development of the biostimulants market by facilitating industrial exchanges and partnerships.

Conclusion

Today, our understanding of plant physiology is more advanced than ever, thanks to significant scientific and technological progress across various disciplines in recent decades. Most of these achievements have been based on studies using a limited number of model organisms under controlled conditions. The current challenge is to apply this knowledge and the associated tools to characterize biostimulants and assess their effects on a wider range of cultivated plants. High-throughput plant phenotyping platforms have been developed to characterize mutants in functional genomics studies. However, there is an urgent need to accelerate research focused on understanding the mechanisms of action of biostimulants, as well as their interactions with environmental stressors and different plant genotypes. Bridging the gap between laboratory data on individual biostimulants and field data on mixtures often combined with fertilizers-remains both difficult and crucial. While root growth promotion by soil bacteria is often clearly demonstrated under laboratory conditions, these results do not always translate into consistent benefits in real-world agricultural settings. The development of biostimulants can follow a classical "pharmacological" approach, wherein potential active substances or microorganisms are screened under controlled conditions, and the most promising candidates are gradually transitioned to more realistic environments. Although effective, this methodical approach involves substantial development costs, which are rarely justifiable in low-margin markets like agriculture and plant nutrition.

Soil microbiologists and ecologists have observed that even during the growing season of annual crops, individual plant varieties interact differently with rhizosphere bacteria, influencing the composition of microbial populations. These genotype-dependent changes in the rhizosphere microbiome may significantly impact plant growth and development. Such findings offer a valuable starting point for understanding successful interactions between plants and plant growth-promoting rhizobacteria (PGPR). From a practical standpoint, new commercial strategies are emerging that aim to amplify the activity of locally beneficial microbiota, rather than relying solely on standardized microbial inoculants. This shift is driven by the recognition that a major limiting factor in the effectiveness of microbial biostimulants is the ability of the introduced organisms to establish and sustain activity within the rhizosphere. The

effective use of biostimulants in agriculture and horticulture requires solutions that are tailored to local environmental conditions and specific timeframes. Tools are needed to monitor the efficacy of biostimulants, and proper management plans must be developed to optimize their application. Additionally, the long-term impacts of biostimulants on ecosystem services and biogeochemical cycles should be assessed and integrated into on-farm decision-making. For biostimulants to realize their full potential, companies must contribute to integrated agricultural solutions at the system, farm, and landscape levels where biostimulants are just one part of a broader sustainable production strategy. Collaboration among stakeholders including farmers, public researchers, industry players, and regulatory bodies is essential to unlock the benefits of biostimulants for profitable and sustainable crop production to support the needs of a growing global population.

Declarations

Competing Interests

The author has declared that no competing interests exist.

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

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