



REVIEW ARTICLE

# Biostimulants in protected cultivation: Unlocking growth potential in horticultural crops

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## Abstract

Plant biostimulants are materials or microorganisms with high nutrient contents that are applied to plants to promote seed germination, stimulate growth and maturation and increase nutrient use efficiency. Biostimulants such as humic acid, seaweed extracts, protein hydrolysates, microbial formulations and inorganic biostimulants are used to increase plant growth, nutrient absorption and stress tolerance. Humic biostimulants enhance the nourishment of roots, while protein-derived biostimulants affect nitrogen uptake and the assimilation process. Microbial stimulants can act through various direct and indirect mechanisms, whereas seaweed biostimulants increase microbial activity, improve nutrient uptake and promote plant growth and soil health. By improving nutrient use efficiency, enhancing stress tolerance and promoting overall crop quality, biostimulants offer sustainable alternatives to conventional inputs. Biostimulants are predominantly used for high-value horticultural crops to improve flowering, yield, quality and shelf-life. Many vegetable growers face challenges due to adverse weather conditions, leading to the adoption of protected cultivation as a high-intensity method to enable year-round production. Biostimulants have been proposed as an effective strategy to promote ecofriendly agriculture, alleviate biotic and abiotic stresses under protected cultivation and reduce the cost of chemical inputs. This review describes the types and functions of biostimulants and their effects on major horticultural crops, with an emphasis on their applications in structured cultivation systems.

**Keywords:** biostimulants; humic acid; horticulture crops; protein hydrolysates; protected cultivation

## Introduction

In recent years, agricultural soil has been found to be severely degraded worldwide. In particular, the fertility, production potential and biodiversity of approximately 40 % of the world's agricultural soil and 24 % of its productive areas have been lost. The main causes of these phenomena include a variety of elements, including salinity, loss of organic matter, wind and water erosion and environmental degradation (1). Over the 40 years from 1959-1999, the global population grew from 3 billion to 6 billion, effectively doubling. It is now expected to increase by 50 % over the following 40 years, reaching 9 billion by 2037. The increasing population, coupled with decreasing arable land and the diminishing genetic potential of crops, underscores the need to adopt innovative agricultural technologies. Agronomic practices with low environmental impact that increase plant resistance to unfavourable soil conditions have become essential to satisfy the rising need for nutritious foods (2).

The European Union introduced the Farm to Fork Strategy (F2F) in May 2020, intending to make it climate-neutral by 2050. This initiative marks an interesting turning point for conservation in agricultural areas. The importance of establishing a healthy and resilient food system that can function

under any circumstances and ensure that residents have access to a sufficient supply of priced food has been made evident by the COVID-19 pandemic. To meet these objectives, F2F strategy aims to maintain food systems that are fair, hygienic and environmentally friendly while, accelerating the transition to a more productive and sustainable food system that uses fewer pesticides, antibiotics and fertilizers. Furthermore, F2F supports increasing the area of land utilized for organic farming to preserve soil health and reduce biodiversity loss (3).

The size of the worldwide horticulture market was valued at USD 22.42 billion in 2022 and is projected to grow at a compound annual growth rate (CAGR) of 9.9 % from 2023 to 2031, reaching USD 52.43 billion. There is an increasing demand for agricultural products to meet the expanding needs of the global population and the growing acceptance of sustainable horticultural methods is credited with the market's growth (4). The overall horticulture production for 2022-2023 is estimated to reach 351.92 million tonnes, which is greater than the total food grain production of 329.69 million tonnes for the same period. India is currently the world's second-largest producer of fruits and vegetables. The nation leads the world in the cultivation of several crops, including okra, papaya, bananas and citrus (5).

Crop-growing systems must be modified to sustain increased food supplies while reducing the adverse environmental impacts of agricultural production. Protected cultivation works best by monitoring temperature, radiation and air composition to produce plants and flowers under less-than-ideal conditions. Another agronomic technique is soilless cultivation, which is particularly useful for vegetable crops. This method avoids the use of soil for cultivation and all its associated issues, such as poor quality or contamination and enables the regulation of water and nutrients (6). Regardless of the weather, greenhouse vegetable production is an intensive, technologically advanced farming method that may assure the year-round availability of off-season produce while simultaneously improving the quality of the end products (7).

Biostimulants may offer a viable solution for addressing degraded agricultural areas and the uncertainties posed by climate change. A plant biostimulant is defined as a substance, microorganism, or combination of both that, upon application to seeds, plants, the rhizosphere, soil, or other growing media, function to increase the intrinsic processes of the plant regardless of the nutritional components of the biostimulant. Biostimulants are “a formulated product of biological origin that improve the plant productivity as a consequence of the novel or emergent properties of the complex of constituents and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds”. These are biological products that are based on amino acids, chitosan, seaweed extracts and humic compounds. Many substances, including lipids, proteins, carbohydrates, phytohormones, amino acids, osmoprotectants, antimicrobial compounds and minerals, can be found in seaweed extracts and when added to plant growth supplements, may have biostimulating effects (8).

Over the past 20 years, plant-based chemicals and other naturally occurring bioactive components, such as humic and fulvic acids, have received much attention. Following their entry into plant components and cells, they have physiological effects on active metabolism, signalling and hormonal control of growth and development in plants (9). In 2015, the European turnover for biostimulants accounted for approximately €500 million, representing nearly 30 % of the global market. The most recent analysis indicates that the global market is projected to surpass a turnover of four billion dollars in 2025 (10).

With a focus on horticultural crop species, the ability to withstand abiotic stress is a critical characteristic, as these plants often have a higher market value than field crops. Moreover, they require greater farming inputs and are a good source of various nutrients, including fibre, minerals and carbohydrates, all of which are necessary for a balanced diet. The sustainable cultivation of horticultural plants has been a primary area of investigation in recent years, with a focus on escalating weather fluctuations and shifts in climate patterns, alongside the effects of biological and environmental stresses on crops.

To promote productivity and product quality in a sustainable manner, plant biostimulants are typically applied to high-value crops, primarily greenhouse crops, fruit trees, open-field vegetables, flowers and ornamentals. Innovative techniques for growing horticulture crops include protected cultivation, which is notable for its high yield, effectiveness and

cautious use of pesticides, water and land. With this approach, high-value crops can be grown year-round, even during the off-season, resulting in higher market prices and increased profitability. Hence, protected cultivation has created an interest among farmers when compared to open field conditions. Practicing protected cultivation along with the utilization of biostimulants can pave the way for sustainable crop production. Furthermore, this review describes various categories of biostimulants and their application in horticultural crop production, especially under structured cultivation practices.

### Biostimulants and its types

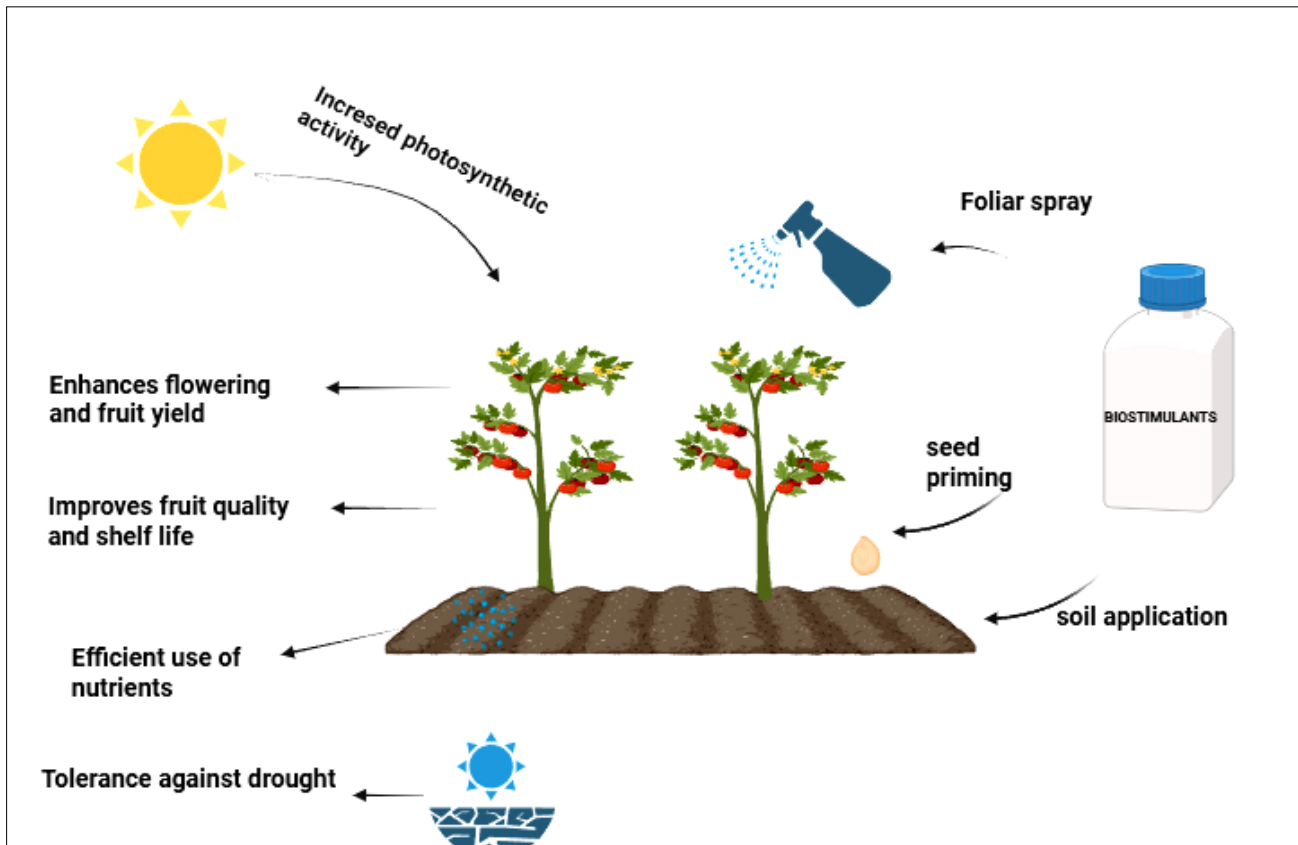
Biostimulants, or biostimulators, are derived from natural compounds that increase plant growth, stress resistance, yield and quality. Their effects depend on their composition, as they provide plants with metabolites, growth regulators and nutrients; however, they are distinct from biofertilizers. Biostimulants are widely regarded as environmentally friendly and key tools in sustainable agriculture. As illustrated in Fig. 1, biostimulants, are typically applied through seed priming, soil application, or foliar spraying. These practices contribute to eco-friendly and efficient agricultural systems (9). Biostimulant application enhances nutrient accessibility, absorption and utilization efficiency as well as resistance to environmental stress, thereby promoting plant growth and yield. Owing to their interaction and function in plant development, they are classified into organic natural substances (e.g., humic and fulvic acids, seaweed extracts and protein hydrolysates), inorganic compounds (e.g., silicon, selenium and copper) and beneficial microorganisms (e.g., rhizobacteria, mycorrhizal fungi and nitrogen-fixing bacteria) (11). Fig. 2 presents the classification and mode of action of biostimulants in various horticultural crops.

### Organic biostimulants

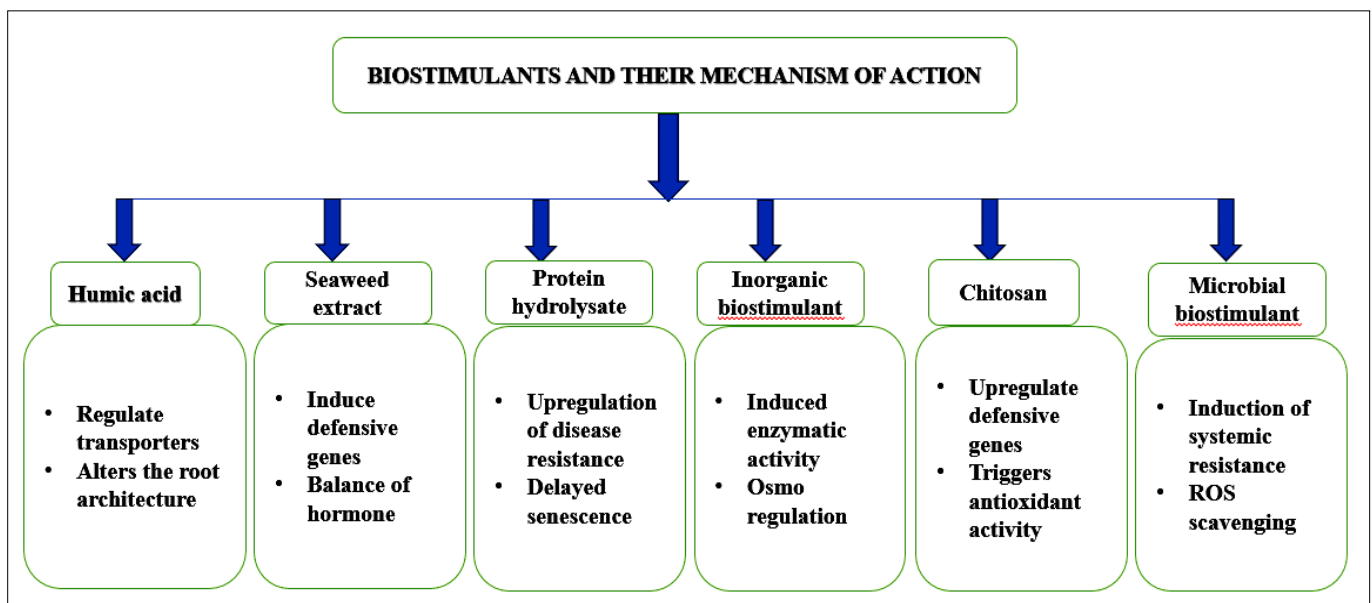
The use of organic plant biostimulants (PBs), which are obtained from natural substances that increase flowering, plant growth, fruit development, crop production and nutrient use efficiency (NUE) as well as resistance to a variety of abiotic stresses, is a promising and environmentally beneficial innovation. Several types of organic waste serve as valuable resources for providing important plant nutrients through bioconversion. The products produced from organic wastes include nutrient-rich supplements and useful energy. Various waste products have demonstrated their efficacy in agriculture and horticulture by yielding biostimulants or organic materials rich in biostimulants. These include vermicompost, humic substances (HSs) and chitin derivatives (12). Since vegetable crops are highly nutrient demanding, organic alternatives to conventional fertilizers are imperative. The application of biostimulants such as humic acid, protein hydrolysates, seaweed extract, chitosan and microbial biostimulants can help achieve balanced plant nutrient levels and improved soil conditions, both of which are necessary for sustained vegetable production.

### Humic substances

Organic materials, referred to as HSs, are formed by microbial metabolism and the chemical and biological modification of decomposing organic matter. They have



**Fig. 1.** Schematic representation of biostimulant applications and their effects on plant growth and productivity.



**Fig. 2.** Classification and mode of action of biostimulants in various horticultural crops.

historically been recognized as crucial elements contributing to soil fertility, significantly affecting the physical, physicochemical, chemical and biological properties of the soil. The biostimulatory impact of these substances is attributed primarily to the increase in root nutrition through a variety of mechanisms, including the increased uptake of both macronutrients and micronutrients (13). Humus, the primary component of HSs, is produced by microbes in the soil by fermenting plant and animal waste in a series of processes under extremely controlled conditions of temperature, time, water, aeration and humidity (14). Humic compounds are renowned for their ability to stimulate plant growth because of their special molecular structure and chemical attributes. The root

serves as the primary focus of HS, exhibiting early differentiation and increased elongation at the root tip and within the primary growth zone (15). HS can affect plant nutrient acquisition strategies in addition to modifying the root anatomy and system architecture traits to optimize soil exploration and nutrient interception. Moreover, HS can affect the regulation of transporters involved in nutrient uptake, increase organic acid exudation from roots and support plant relationships with beneficial rhizosphere microorganisms (16) and stabilize soil aggregates, thus preventing soil loss by leaching. When plants ingest humic compounds, various molecular processes in their cells are triggered, increasing their resistance to multiple abiotic stresses.

When humic acid is applied to plants, it increases the expression of the H<sup>+</sup>ATPase enzyme, which stimulates root growth analogous to the action of the auxin hormone (17). In tomato (*Solanum lycopersicum*) and okra (*Abelmoschus esculentus*) plants, increased salinity tolerance has been demonstrated (18). The benefits of HSs have been shown in various horticultural crops and are listed in Table 1.

**Table 1.** Effects of humic substances on various horticultural crops.

Crop	Effect of humic substances	Reference
Potato	Increase the early yield of approximately 2.6 % potato when cultivated in low temperature	(19)
Melon	Reduce the adverse impact of excessive salt in melon plants	(20)
Garlic	Improved yield and quality of bulbs	(21)
Onion	Increased plant growth and yield	(22)
Chilli	Increased capsaicin content	(23)

### Protein hydrolysates

Protein hydrolysates (PHs) are a class of organic biostimulants derived from plant and animal sources of proteins. They have gained importance because of their interesting role in crop production, covering seed germination, yield improvement and quality enhancement in various agricultural and horticultural crops (24). They are composed of polypeptides, oligopeptides and amino acids produced by the partial breakdown of protein resources. PHs are categorized based on the source of the protein (plant or animal source) and the method used to hydrolyse it. Some of these methods involve chemical, thermal or enzymatic hydrolysis, which influences the chemical properties of proteins (25).

PHs increase a plant's ability to withstand harsh climatic factors, especially by increasing the plant's antioxidant activity (24). They may also influence the plant directly by modifying its nitrogen and carbon metabolism and altering its hormonal profile or indirectly by influencing the associated microbiome. When PHs are present at the leaf or root level, the microbial community occupying the phyllosphere or rhizosphere is altered (26). Furthermore, by increasing plant accessibility to mineral nutrients, microorganisms in the rhizosphere and phyllosphere may release enzymes that further breakdown the peptides into smaller pieces, which function as signalling molecules to promote plant growth (24). PH-based biostimulants have major applications in horticultural crops. It is believed that some amino acids and peptides have chelating and complexing properties that enhance nutrient availability and nutrient uptake by roots. These compounds influence plant development by balancing phytohormones (27), increasing the antioxidant properties of treated plants and elevating the levels of phytochemicals such as carotenoids, flavonoids and polyphenols.

Several recent studies highlighted the benefits of using biostimulants made from PHs of agro-industrial byproducts to promote the potential of horticultural crops to grow, yield and perform under different abiotic stresses.

PH-based biostimulants have been extensively studied for their ability to alleviate the effects of drought stress when applied at the leaf level. However, they appear to be more successful in promoting plant growth under stressful circumstances, particularly when applied through fertigation (28). Its application to either roots or foliage derived from diverse sources has been shown to stimulate root development and alter root architecture. Additionally, they improve nutrient uptake by stimulating the function of key enzymes, such as nitrate reductase, glutamine synthetase and Fe (III)-chelating reductase, while also increasing resistance to both biotic and abiotic stresses (29). Research has demonstrated that PH can activate or enhance immune responses against abiotic stress. For example, in tomato plants, applying a PH biostimulant reduced the storage of toxic ions (Na<sup>+</sup> and Cl<sup>-</sup>) while increasing the levels of beneficial cations (K<sup>+</sup> and Ca<sup>2+</sup>) in the roots and leaves. This treatment mitigated the effects of excessive salt in the soil and irrigation water. The activities of antioxidant enzymes (catalase, glutathione reductase and superoxide dismutase), proline content and oxidative stress markers (malondialdehyde and hydrogen peroxide) are significantly greater in plants treated with biostimulants, thus reducing the degree of oxidative, ionic and osmotic stress in plants exposed to high salinities (30).

In tomato, the enzymatically hydrolysed animal protein-based biostimulant peptone has beneficial effects on primary and lateral root growth through the stimulation of the production of hormonal pathways, such as the salicylic acid pathway, when plants are grown under stress (31). Numerous studies have highlighted the positive impact of PHs on plant physiological processes, contributing to improved yield and quality traits. Therefore, PHs have significant potential for enhancing nutrient uptake and improving stress tolerance, thereby increasing the overall crop performance. The effects of PH on various horticultural crops are given in Table 2.

**Table 2.** Effect of protein hydrolysate on various horticultural crops.

Crop	Effect	Reference
Common bean	Maintained plant growth, yield and mitigated negative effects of salt stress upto 7.8 dS m <sup>-1</sup>	(32)
Lettuce	Mitigation of oxidative and salt stress and increased glucosinolate content, exhibit most favourable metabolomic profile	(28)
Apple	Enhances sensory and overall fruit quality	(33)
Grapevine	Mitigate the water stress	(34)

### Seaweed-derived biostimulants

Seaweed extract is another popular natural biostimulant that improves crop quality, abiotic stress tolerance and nutrient efficiency in plants. It also has beneficial effects on soil microbes, soil retention, remediation and possible nutrient supplies. Seaweeds are macroalgae that play an essential role in marine and coastal environments, contributing to the diversity of these ecosystems and to the biosphere. Most importantly, approximately 10,000 species of seaweed, also known as macroalgae, are generally

divided into three groups according to the pigmentation of the algae: Phaeophyta (brown), Rhodophyta (red) and Chlorophyta (green). Brown seaweeds such as *Ascophyllum*, *Fucus* and *Laminaria* are the dominant groups widely used in the development of biostimulants (13).

Owing to their distinct bioactive components and significant potential, seaweed-based ecological substances have become increasingly popular in crop production systems. The phytostimulatory qualities of these plants lead to higher yield characteristics and plant growth in several significant agricultural plants. Since their constituents allow plants to induce defence mechanisms against a variety of diseases, pests and abiotic conditions such as cold, salinity and drought, they are considered to have phytoelicitory effects (35).

Seaweed extracts have been used in most vegetable crops for a wide range of advantages, such as enhanced germination in beans (36), early flowering and increased fruit development in a range of crop plants, viz., snap beans, tomatoes and peppers (37). The application of seaweed extracts eventually improved yield by increasing flower count and fruit set. Tomato crops treated with seaweed extracts presented considerable increases in the number of flowers and inflorescences, the flower-to-fruit ratio and the quantity and size of fruits (38). The increased yield is believed to be caused by the different concentrations of phytohormones observed in the extracts, especially cytokinins and the activation of host hormonal production. Seaweed extract has been widely used for enhancing the yield characteristics in several crop plants and recent studies reporting its beneficial impacts on horticultural crops are presented in Table 3.

**Table 3.** Effects of *Ascophyllum nodosum* extract on various horticultural crops.

Crop	Effect	Reference
Spinach	Increase in yield and nutritional quality	(39)
Strawberry	Improved plant growth and increase in yield upto 26 % on an average.	(40)
Common bean	Alleviate the water stress effects on plant	(41)
Tomato	Induce flowering and fruit set	(37)
Broccoli	Improves the yield and nutritional quality	(42)

### Chitosan

Chitin, a biopolymer naturally found in fungal cell walls, insect exoskeletons and crustacean shells, is deacetylated and transformed into chitosan. Chitosan is widely used in a variety of industries and fields, including agriculture, medicine and allied industries. Chitosan and its related biopolymers have been shown to exhibit biostimulatory action in a variety of crops, particularly in vegetable species susceptible to stress, where chitosan application can be extremely beneficial (11). The application of chitosan as a seed coating has the potential to increase germination rates and seedling development by triggering antioxidant activity or by facilitating water absorption via the development of a semipermeable coating on the seed

surface (43). Currently, chitosan appears to be the most cost-effective method to increase the quantity and quality of a variety of plants, particularly those with considerable value, such as horticultural crops (44). Recent reports demonstrating the beneficial effects of chitosan on different horticultural crop species are presented in Table 4.

**Table 4.** Effects of chitosan on various horticultural crops.

Crop	Effect of chitosan	Reference
Basil	Chitosan increases total phenolic content of approximately 20.7 mg g <sup>-1</sup> and antioxidant activity also reduce the negative effects of dryness	(45)
Ginger	Chitosan and oligochitosan increased the activity of resistance enzymes.	(46)
Bell pepper	A chitosan nanocoating extended the shelf life of fresh cut bell peppers	(47)
Pepper	Plant morphology, growth and physiology improved by nano chitosan	(48)
Potato	Chitosan prevented <i>Phytophthora infestans</i> growth and spore germination	(49)

### Inorganic compound-derived biostimulants

The chemical components that can promote plant growth without being necessary for all plant species are referred to as efficient factors (13). Selenium and silicon, along with cobalt, aluminium and sodium, are the primary beneficial elements. These elements exist as inorganic salts in both soils and plants, including insoluble salts such as the amorphous silica found in grasses. Two types of positive effects can be induced: constitutive effects, such as silica deposits reinforcing cell walls or temporary effects, such as exposure to specific abiotic stimuli.

Numerous positive effects associated with beneficial elements, have been demonstrated, including increased plant growth, improved plant product quality and increased resistance to abiotic stress. These outcomes are linked to various physiological and biochemical processes, such as cell wall strengthening, osmoregulation, decreased transpiration due to crystal deposits, temperature regulation through radiation reflection, co-factor-induced enzyme activity, plant nutrition through elemental interactions during uptake and mobility, antioxidant defence, symbiont interactions, pathogen and herbivore responses, protection against the toxicity of heavy metals and plant hormone synthesis as well as signalling mechanisms (50).

A crucial factor in determining the suitability of a silicon source for agricultural use is its heavy metal content. It must be low enough to prevent excessive accumulation in the soil and uptake by plants, which could pose risks to both crops and consumers. Soluble silicates have successfully addressed these concerns, establishing themselves as widely used industrial compounds in agriculture, particularly as biostimulants and fertilizers (51). Among them, potassium silicate is the most utilized bioactive material for mitigating abiotic stress and contributing to plant disease management. Notably, it has been recognized as safe by the United States Food and Drug Administration (FDA) (52). Although potassium silicate is applied topically and in hydroponic nutrient

solutions, its cost prohibits its usage in soil at quantities sufficient to function as a biostimulant and increase silicon uptake by plants silicon. If the Si product in use releases enough soluble silica, application through the soil is far more powerful than foliar treatment for increasing silica levels in plant parts and producing stimulatory effects. The most widely used sources of silicon in horticulture for soil application include naturally occurring materials such as olivine, wollastonite and diatomaceous earth, as well as a variety of steel industry byproducts such as silica fume, steel slag, blast furnaces, ferro manganous slag and converter slag (51). The application of these elements as biostimulants resulted in increased growth and tolerance to multiple stresses in various crops (Table 5).

**Table 5.** Effects of inorganic biostimulant compounds on various crops.

Crop	Effects	Reference
Onion	Selenium - mitigate the abiotic stress and increase the yield of approximately 25 %	(53)
Tea	Selenium - promote growth quality of leaf and stress resistance	(54)
Strawberry	Silica - Improves the yield	(55)

#### Microbial biostimulants

The application of microbial plant biostimulants (PBs) has emerged as a promising substitute for the use of agrochemicals (less demand and reduced use of pesticides in integrated farming systems) and as an ever-interesting area of research. The positive benefits of microbial biostimulants have been proven thus far, largely through their mutual relationships in the environment of plant-soil microbes, which are regarded as a biological tool to increase the health parameters of several horticultural crops and to reduce soil degradation (56).

The addition of beneficial microorganisms can alter the biological composition of the soil, increase the population of symbiotic microbes that increase soil health and crop output and support the restoration of native soil microbes (57). Additionally, they may influence water availability in the root region; modulates root ethylene and auxin levels; regulates shoot responses through the control of stomata and xylem hydraulic conductivity and impact the entire plant responses through ROS scavenging, membrane stability and osmoprotection (18).

The four distinct genera of microorganisms, *Azotobacter* sp., mycorrhizal fungi, *Rhizobium* sp. and *Azospirillum* sp., constitute microbial plant biostimulants. Arbuscular mycorrhizal fungi (AMFs) and plants are capable of symbiotic interactions that increase the root surface area required for nutrient intake (58). A group of plant growth-promoting microorganisms (PGPMs) can also act as plant biostimulants

The proposed mechanism of action of PGPMs on plant growth includes (i) hormonal control; (ii) balancing of cell oxidative conditions; (iii) increasing water use efficiency and photosynthetic metabolic responses and (iv) increasing nutrient use efficiency (59). PGPR (plant growth-

promoting rhizobacteria) positively impact plant growth by the production and regulation of certain substances, such as phytohormones and by increasing the accessibility of essential nutrients, such as nitrogen, phosphorus and iron. Moreover, they can interact directly with plants (60).

In tomato crop production, the combined effects of *Trichoderma*, mycorrhizal fungus and nonmicrobial PBs such as vegetal extracts have been studied. The ability of AMF and *Trichoderma* to release auxin-like chemicals into the rhizosphere has been shown to promote tomato crop growth. Through antagonistic activity and the development of systemic resistance in plants, they can suppress plant diseases and solubilize mineral elements such as phosphorus and micronutrients (61). PGPB and AMF combined with *Trichoderma* improved tomato yield, fruit count and the concentrations of key amino acids,  $\gamma$ -aminobutyric acid, mono ethanolamine (MEA) and secondary metabolites that act as antioxidants, such as polyphenols and lycopene, in tomato. Owing to their ability to affect plant physiological mechanisms and increase both production and quality, microbial biostimulants may provide a viable and environmentally friendly solution (62). Recent studies on the responses of various horticultural crop species to microbial stimulants are presented in Table 6.

**Table 6.** Effect of microbial biostimulant on various horticultural crops.

Crop	Effect	Reference
Shallot	41.37 % increased germination when seeds are treated with PGPB biostimulant.	(63)
Coriander	Seed inoculation with <i>Azotobacter chroococcum</i> and <i>Azospirillum lipoferum</i> increased biomass production	(64)
Tomato	Improved the fruit quality with the application of AMF and <i>Trichoderma</i> .	(65)
Lettuce	Lettuce seeds inoculated with <i>Azospirillum</i> exhibited improved germination rates and enhanced vegetative growth.	(56)

#### Combined effects of biostimulants

In addition to major categories of biostimulants, some researchers have evaluated the combined influence of different biostimulants. When biostimulants are applied in combination, these components have a synergistic effect on crops, providing a stronger root system, promoting plant growth and improving leaf development and flowering. The results of recent studies are presented in Table 7.

**Table 7.** Combined effect of biostimulants on horticultural crops.

Combination	Crop	Effect	Reference
Microalgae extract + Humic substance	Onion	Improved growth and 34 % higher yield	(66)
PGPB + humic acid	Potato and tomato	Increased plant growth	(67)
<i>A. nodosum</i> + Humic acid	Lettuce and spinach	Enhanced plant growth and shelf life	(68)
PGPR + seaweed extract ( <i>E. maxima</i> )	Amaranthus	Increased the photosynthetic pigment content	(69)

## Response of biostimulant in ornamental crops

The cultivation of ornamental plants is one of the fastest-growing segments of the horticultural industry, particularly about manufacturing ornamental potted plants, which are becoming increasingly popular around the world. For the vegetative propagation of ornamentals, several methods have been employed. The most common method for multiplying clones of woody and herbaceous landscape plants is stem-cutting propagation, which is less costly and simpler than the "*in vitro*" method (70). The ornamental nursery industry continues to encounter challenges with insufficient rooting efficiency despite significant advancements in stem-cutting propagation, a difficult process that begins with excellent-quality rooted cuttings. Nevertheless, synthetic phytohormones such as IBA, which has a large carbon footprint, increase adventitious root synthesis when administered externally to cuttings (71).

Biostimulants are useful tools for optimizing the propagation efficiency of vegetative cuttings, but their ideal application rates are frequently species specific. When a plant biostimulant is applied to Rosa 'Hurdal' cuttings, the phloem tissues of rose plants thicken and further applications cause the xylem cells to enlarge, strengthening the stem and promoting the proliferation of plant roots (72). The use of microalgae extracts promote physiological processes such as photosynthesis, nutrient metabolism, enzyme activity and chlorophyll and carbohydrate content in cuttings thereby enhancing the rooting process (73). In the cultivation of ornamental bedding plants, biostimulants are widely applied. The growth of *Helianthus annuus* L. seedlings, which are also employed as annual ornamental bedding plants, is improved by *Ascomyces nodosum* algal extract, which also lowers the cost of seedling production (74).

The application of plant extracts, vegetable-derived PHs and seaweed extracts improved the yield of wild rocket (*Diplotaxis tenuifolia*) without increasing the nitrate content under organic farming conditions, when the stimulants were applied individually as foliar sprays at weekly intervals (75). *Anthirrinum majus* L. and petunias are significant cut flowers in the ornamental plant trade (76) and are grown commercially under greenhouse conditions. The application of biostimulants, especially the use of PHs as leaf sprays, has improved the production of high-quality plants.

Humic acid is also used in ornamental horticulture as a biostimulant and enhances vegetative and floral growth. The corm properties of gladiolus are significantly impacted by the utilization of humic acid. In the case of cut flowers, humic acid, fulvic acid and seaweed extract can also be used to extend the vase life and increase the number of blossoms in woody-stemmed plants (77). The application of the commercial biostimulant Biovita at 0.6 % under field conditions resulted in increased synthesis of photosynthates by promoting vegetative growth, which may have led to an increase in flower production as well as the quality (78) in commercial chrysanthemum cultivation. These motivated the growers to employ Biovita for the yearly production of flowers.

The effects of different biostimulants were also studied in the absence of soil cultivation. The influence of three various agricultural biostimulants, viz., an animal-derived PH biostimulant, a vegetal-derived PH biostimulant and an algal-derived PH biostimulant, on lily hybrids cultivated in a soilless environment with respect to decorative flower crops, yielded the greatest increase in leaf area and flower bud count under the application of animal-derived PH (79), when compared to the other sources. The application of animal-based PHs to potted Petunia cultivation by foliar spraying and root drenching had the greatest effect on root morphology, dry biomass and leaf N, P and K contents and assisted in the production of extragrade plants (76).

Chitosan is a comprehensive biostimulating alternative for the commercial propagation of orchids, offering excellent biocompatibility and biodegradability. It is also a very effective category of biostimulants, especially under micropropagated orchid culture. When combined with coconut water, it positively enhances the growth of the orchid *Cattleya maxima* (80). Soaking *Eucomis bicolor* bulbs (Baker bulbs) in a chitosan solution Prior to planting increased development, blooming and yield. At the optimum concentration of chitosan, the number of leaves per plant, the relative chlorophyll content of the leaves and the number of bulbs per plant increased (81). Hence, biostimulants play a major role in ornamental plant production by increasing the percentage of germination and the resistance of plants to various biotic and abiotic stresses until harvest and post-harvest. Proper dosages of biostimulants must be used to increase plant growth and development.

The existing literature emphasizes the potential of biostimulants in the production of ornamentals. Ornamental crops are very much considered for their aesthetic value. In general, the use of biostimulants improves seed germination and stimulates root formation in cuttings, which might be due to the synthesis of growth hormones. Growth hormone production is believed to be influenced by the presence of precursor amino acids. This benefit offered by biostimulants helps replace the application of growth hormones such as IAA and IBA, which offer other benefits, such as improved propagation efficiency, bulb development, blooming and increased chlorophyll content, thereby improving the generation of photosynthates. These stimulants protect plants from abiotic and certain types of biotic stress by increasing plant fitness through the enhancement of nutrient and water uptake and growth characteristics. They have been reported to promote the shelf life and vase life of flowers as well as improve flower color and quality. This effect is highly important in the development of micropropagated orchid plants. Given these benefits, the application of biostimulants should be encouraged in the large-scale cultivation of flowering crops, which reduces cultivation costs and provides long-term sustainability.

## Effects of biostimulants on major horticultural crops under structured cultivation

One of the critical global challenges in the twenty-first century is climate change, which is caused mostly by the increasing concentration of greenhouse gases in the

atmosphere. Global climate change has a drastic effect on both the quantity and quality of sustainably grown horticultural crops. Climate change adds on to environmental stress on crops by altering temperature, changing patterns of precipitation and increasing the incidence of pests and diseases (82). India experiences climatic challenges that lead to reduced crop production and significant economic losses to farmers. To protect plants from these challenges, protected cultivation is being adopted by farmers. Additionally, this type of cultivation enables year-round production, facilitating off-season cropping.

A better method of cultivating crops in a regulated environment is called protected or greenhouse farming. Crops are protected from biotic (diseases and insect pests) and abiotic (temperature, rain, wind, humidity, etc.) stresses. These variables can be controlled to meet the needs of the crop. In protected cultivation systems, the natural environment is modified by changing practices and structural components to achieve the best possible yield of crops. Vegetables and flowers can be cultivated year-round despite unfavourable weather conditions by establishing an appropriate microclimate. Off-season cultivation not only promotes year-round crop production but also results in higher costs compared to seasonal cropping. For the protected cultivation of vegetables and cut flowers, greenhouses or poly house cultivation are frequently used. Commercial greenhouse operations facilitate the production of exotic (nonnative) and off-season crops, export-quality cut flowers and high-quality seedlings. An increase in high-value agricultural produce in a greenhouse can result in increased economic returns. (83). Protected cultivation enables the production of flowers with better quality, including a larger diameter, longer stalk, color stability and superior quality. Therefore, protected cultivation is considered the most viable approach to attain year-round production. It ensures the availability of out-of-season goods all year round, despite the weather, while also improving the quality of the finished product (84). Fig. 3 illustrates the diverse effects of biostimulants on vegetable production under structured cultivation conditions, highlighting their roles in enhancing plant growth, improving stress tolerance and promoting physiological and metabolic processes.

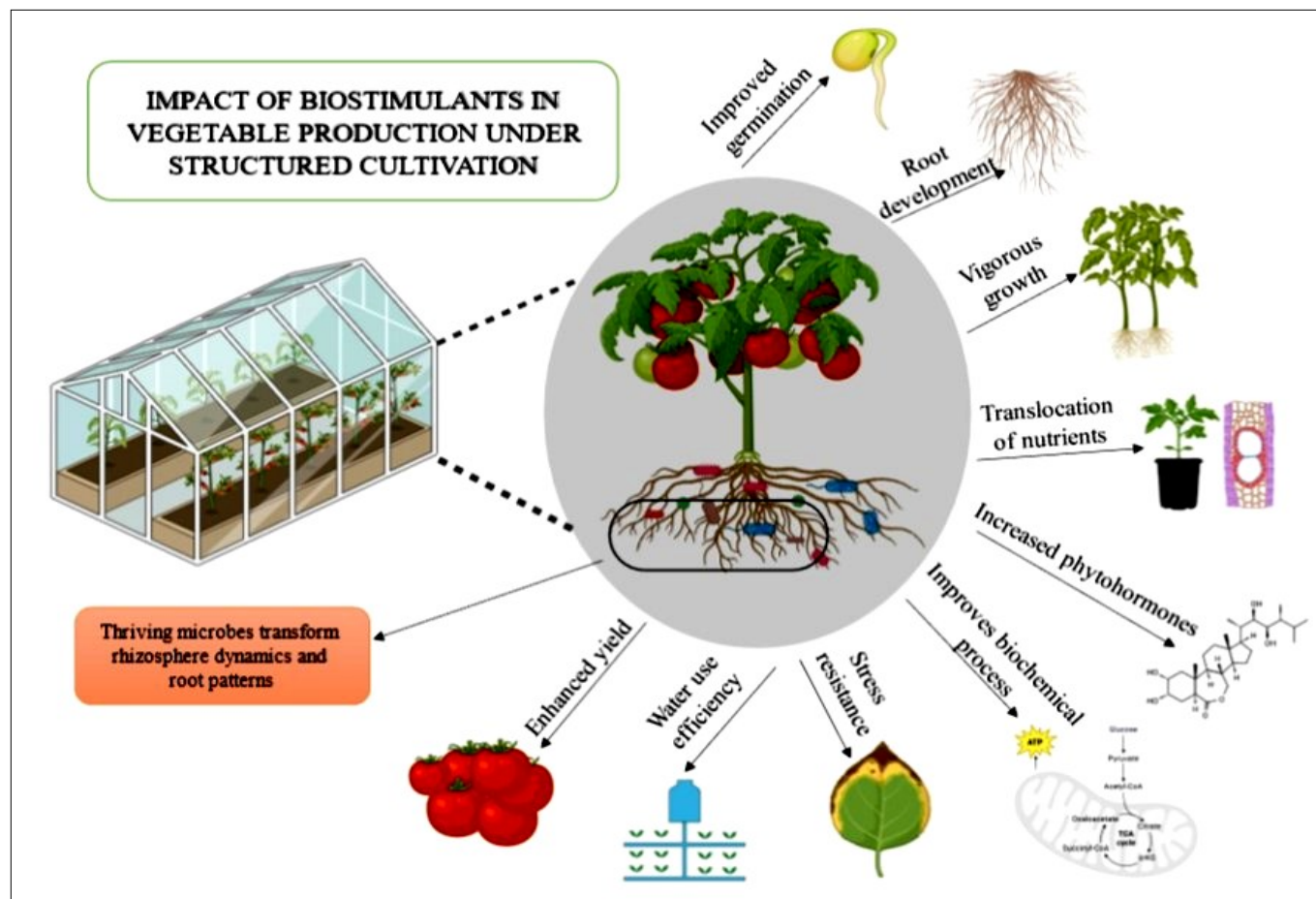
Protected farming effectively supports the cultivation of major vegetable crops, including capsicum, beans, cauliflower, tomato, cucumber, pea, leafy and exotic vegetables. Flower crops grown under protected farming include gerbera, orchids, lily, anthurium, carnation and rose (83). These poly house cultivations provide a platform for increasing the need for plant biostimulants. Since greenhouse growers and industries are interested in accelerating the growth of crops cultivated in greenhouses in a sustainable manner, the use of biostimulants in high value-added crops such as vegetables under intensified cropping systems is warranted (85). Most investigations under structured cultivation have focused on the use of biostimulants such as brown macroalgal extracts and PH to achieve good crop

standing and productivity, especially in vegetables and ornamentals.

Tomatoes (*Solanum lycopersicum* Mill.) known for high concentrations of health promoting compounds, particularly vitamin C and lycopene, are among the most significant vegetable crops grown under protected culture conditions globally and constitute an essential part of the human diet (86). It has been reported that biostimulants can not only increase the yield but also reduce the negative effects of high temperatures on greenhouse-grown tomatoes. Tomatoes can be produced year-round in greenhouses, but the primary drawback is the extreme heat within these kinds of structures. However, application of biostimulants can help alleviate heat stress, thereby increasing overall plant performance (26). Despite rising production costs, farmers' net revenue has increased significantly, due to the use of biostimulants which has improved crop performance and fruit nutritional indices in greenhouse-grown tomato plants (84). Ascorbic acid and total phenolic compounds presented positive responses in plants treated with biostimulants. This technology may have a direct effect on phytochemical homeostasis (86) or may have an indirect effect due to the increased concentrations of K and Mg, which may stimulate antioxidant synthesis in in greenhouses grown vegetables.

When algal extracts were applied topically to greenhouse-cultivated tomatoes at high temperatures, yields were greater than those found in the control group. Moreover, it mitigated the effects of heat stress, promoting a greater number of fruits with greater fruit mass, possibly due to reduced fruit abortion, improved fruit retention and a decrease in lost fruits per plant. Additionally, the hormonal effects of biostimulants regulate the expression of genes involved in tomato fruit development, leading to increased yield (87). Furthermore, the increased yield, observed in tomato plants treated with plant extracts, seaweed extract and PH coincided with enhanced quality traits. The application of legume-derived PH increased the qualitative features of fresh tomatoes in terms of TSS, lycopene, K and Mg concentrations (26).

Recent studies on microbial biostimulants in cucumber cultivation have yielded promising results. PGPR biostimulants comprising six species of *Bacillus* (*B. subtilis*, *B. pumilis*, *B. megaterium*, *B. amyloliquefaciens*, *B. velezensis* and *B. licheniformis*) applied through seed priming of zucchini squash cultivated under both greenhouses and open field conditions significantly increase nutrient levels, biomass and fruit production (88). Similarly, the application of bacterial-based biostimulants along with seaweed extract significantly increased the plant growth, yield and fruit quality in cucumber, thereby maintaining a superior balance between both quantity and quality of cucumber (89). Inorganic biostimulants also have a good response to greenhouse cultivation. Foliar application of silica to zucchini cultivated under greenhouse condition enhances plant growth by stimulating various biochemical pathways, including those involving pathogen-related pigments, flavonoids, antimicrobial enzymes and antioxidants (90).



**Fig. 3.** Effects of biostimulants on vegetable production under structured cultivation.

Green leafy vegetables are the most nutritious produce containing higher levels of vitamins, minerals, iron and calcium. Hence, cultivation under protected systems results in greater yields than cultivation under open conditions. More pronounced effects of the biostimulants were observed in the greens. A recent study highlighted the potential benefits of biostimulants, viz., seaweed extract (extracted from *Ecklonia maxima* and a combination of vegetable oils, herbals and seaweed extracts of *Ascophyllum nodosum*) and a legume-derived PH in spinach. Foliar sprays of these biostimulants led to increased leaf size, higher chlorophyll content and increased photosynthetic ability. These effects were associated with changes in root structure that promoted nutrient uptake and increased phytohormone synthesis, resulting in increased biomass production and yield. The biostimulant effect also had a favourable effect on the composition of minerals (K, Mg and Na) and proteins. The improvements in nutrient uptake observed in green leafy vegetables are attributed to the presence of signalling molecules in biostimulant products, the modifications made in root architecture and the increased regulation of genes involved in macronutrient transport. These factors collectively contribute to the protein and macronutrient content in green leafy vegetables (86).

A previous study aimed to evaluate the effects of biostimulant application on the nutritional value and bioactive properties of spinach grown under water stress conditions in a controlled environment. Four commercially available biostimulants-Megafol (MEG), Aminover (AM), Veramin Ca (V) and Twin Antistress (TA)-were tested on

two spinach genotypes, Fuji F1 and Viroflay, under two irrigation regimes: water-stressed (W-) and regular irrigation (W+). The findings revealed that the bioactive properties and chemical composition of both genotypes responded differently to the biostimulant and irrigation treatments. Water stress increased the carbohydrate and fatty acid contents in Fuji plants treated with Megafol (MEGW+) and Veramin (VW+), with MEGW+ resulting in a higher caloric value. In contrast, the protein and ash contents were elevated in Viroflay plants treated with AMW- and TAW+. The predominant fatty acids identified in spinach are linoleic acid and  $\alpha$ -linolenic acid (91).

Foliar application of seaweed extracts has been shown to increase plant growth and productivity, especially under suboptimal nitrogen (N) regimes. Baby leaf lettuce cultivated in an unheated plastic tunnel system showed a positive response to biostimulant application at N application rates of 20 and 30 kg ha<sup>-1</sup>. The improved agronomic performance was attributed to the ability of the biostimulants to increase physiological and biochemical parameters, such as the SPAD index and chlorophyll and carotenoid contents. This study highlighted the benefits of using seaweed-based biostimulants in increasing the yields of baby lettuce under both optimal and deficient N conditions, providing advantages for both farmers and the environment (92). Among the various agronomic claims associated with plant biostimulants, their capacity to improve nitrogen use efficiency (NUE) stands out as a key factor driving their commercialization for both environmental and economic benefits.

In broccoli cultivation under protected conditions, the addition of the algal extract produced the best overall marketable yield, lateral head weight, plant yield and main head weight in addition to the significant influence on nutrient levels (38). In the greenhouse cultivation of woodland strawberry, the application of PH and the microbial stimulant *Trichoderma atroviride* greatly increased fruit weight; total sugars, ascorbic acid, flavonoids and anthocyanin contents as well as antioxidant activity (93).

The main objective of commercial flower cultivation is to achieve a qualitatively and quantitatively greater floral yield. One of the most popular commercial flower crops, chrysanthemum, is farmed for loose and cut flowers. The blooms can be used as bedding plants, decorations for vases, garlands and garden decorations. The fan and pad greenhouse system of cultivation produced better floral features and higher yields when different biostimulants were applied (94). The application of *Ascophyllum nodosum* @ 5 mL L<sup>-1</sup> at 30, 45 and 60 days after transplanting proved to be the best treatment for increasing the flowering, vase life and net return of *Chrysanthemum* cv. Denjigar white grown under naturally ventilated polyhouses. Seaweed extract application induces photosynthesis, which might lead to the recombination of nutrients in flowers and enhance floral longevity, resulting in an increased shelf-life. Additionally, it suppresses ethylene and abscisic acid production, which may prolong the shelf-life and appearance of flowers (77).

India, one of the world's mega biodiversity hotspots, boasts a rich orchid history, with over 1,300 species over 167 genera spread across the country. The use of chemicals in orchid cultivation may have residual effects and affect shelf-life. Biostimulants also play a greater role in soilless cultivation systems. In the cultivation of *Dendrobium* under ventilated greenhouses, the application of seaweed extracts increased plant height, leaf area and yield characteristics. It is caused by specific precursors, such as auxin, cytokinin and other nutrients found in stimulants, which encourage cell growth and multiplication. Improvements in the development of floral characteristics, including spike initiation, have also been observed (95). Anthuriums are widely accepted both as potted decorative plants and as cut flowers. The application of biostimulants had an important effect on the growth and floral characteristics of *Anthurium* and *Solanum* cv. Dora when grown in a naturally ventilated polyhouse. Topical application of 2 % humic acid-fulvic acid mixture every two weeks resulted in the earliest flower bud initiation and the highest values for stem width, leaf breadth, leaf length, spathe length and stalk length, number of flowers, floral duration and vase life (96).

In hydroponic systems of cultivation, biostimulants also play a vital role. Soilless methods, such as floating raft systems, require foreign chemical inputs and are not always ecologically sound. Thus, adding biostimulants to nutrient solutions may be a useful strategy to improve the resilience of hydroponic systems. Hydroponically grown *Ocimum basilicum* (97) yielded more fresh produce when a protein-based biostimulant was added to the nutrient solution. Biostimulant application improves several functional processes, such as the activation of enzymes involved in nitrogen and carbon

metabolism, stimulation of phytohormones and increased mineral intake through roots (6).

### Potential drawbacks of biostimulants

Biostimulants are generally considered beneficial supplements for increasing crop growth, productivity and nutritional parameters. While many of the above studies have supported the benefits at a commercial scale and a few have also highlighted inefficiencies under large-scale application. Compared with thoughtfully organized controls, research on several biostimulant products has revealed that some are ineffective or possess inert, unstable or inconsistent qualities (98). Notably, a product containing amino acids from animals was shown to have detrimental effects on Fe nutrition and severely inhibit plant growth when applied both topically and through the roots, whereas a different product containing amino acids promoted plant growth (99). Another investigation examined several biostimulant products and it was observed that none of the products achieved an adequate level of pathogen management that would suggest their replacement or addition to conventional synthetic fungicides (100).

### Future prospects

Biostimulants are natural products that increase crop growth, productivity and resistance to several biotic and abiotic challenges, thereby reducing the use of chemical fertilizers under normal conditions. During the off-season cultivation, when environmental factors are not suitable for crop growth, cultivating crops under protected cultivation with the inclusion of biostimulants can enhance yield and consistent product availability throughout the entire period. Additionally, it helps minimize the accumulation of chemical residues in food, making it a valuable approach for sustainable agriculture and human health in the future. More studies are needed to elucidate the mechanisms behind the various biostimulant activities and to identify effective and acceptable biostimulants for specific crops as well as their growing conditions. In addition, there is a need to recommend the best appropriate dosage levels and delivery systems for application across different crop stages in both open field and protected cultivation. In India, many biostimulants are on the market, but there are still no definite standards for assessing their quality. Government sectors and policy makers should engage in this field to define the quality of the products. Owing to the lack of stringent controls, various false products are entering the market, leading to mistrust among farmers. Hence, there is an urgent need to develop quality standards for various types of biostimulant products.

### Conclusion

This literature review highlights the significant positive impact of biostimulants on various horticultural crops, positioning them as a sustainable alternative to chemical fertilizers. Every farmer has the right to understand and benefit from such innovations in crop cultivation. Therefore, researchers and extension workers are responsible for defining product quality clearly and raising awareness among farmers regarding the availability, application methods and potential

benefits of biostimulants as a greener approach to agriculture. Despite increasing interest, several research gaps persist. These include the absence of standardized application protocols across different crops and agroclimatic regions, limited understanding of their mechanisms of action under field conditions and a lack of long-term studies on their effects on soil health and crop productivity. Addressing these gaps through focused research is essential for supporting wider adoption and fully integrating biostimulants into sustainable horticultural practices. The effectiveness of biostimulants varies between protected cultivation systems and open-field conditions. Its use, particularly during postharvest stages, has been shown to improve crop yield, quality, shelf life and resistance to biotic and abiotic stresses. To ensure consistent results, the biostimulant industry must provide high-quality, reliable products. In addition to being eco-friendly, biostimulants enhance the efficiency of natural resource use and reduce reliance on agrochemicals, ultimately lowering the carbon footprint and contributing to protect the ecosystem.

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## Authors' contributions

ND contributed to writing the article, KK carried out conceptualization, literature review and correction of the article. KGS, PV, MA and RA reviewed and helped in editing the article. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Bio Render tool to create images and Grammarly to improve the language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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