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BIOSTIMULANTS FOR SUSTAINABLE FLOWER CROP PRODUCTION: A REVIEW

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ABSTRACT
Biostimulants are an emerging sustainable approach to increase the growth, productivity and quality of flower crops. They contain bioactive compounds that enhance plant metabolism, nutrient-use efficiency, and stress tolerance in plants. A wide range of biostimulant products is used in the commercial cultivation of flower crops, such as humic substances, seaweed extracts, protein hydrolases, botanical extracts, chitosan, and microbial biostimulants, which have shown significant positive effects on these crops. This review provides details about the composition and role of each biostimulant effect in vegetative and flowering characteristics. The application rate and effect on commercial flowers are given briefly. In addition to improving plant growth, some biostimulants, such as chitosan and botanical extracts, enhance tolerance to biotic and abiotic stresses. As a result, they contribute to improvements in both quantitative and qualitative traits of flowers, including yield, size, colour, and overall market value. Biostimulant also used to extend the vase life of cut flowers, which helps in reducing the usage of preservatives. Moreover, it induces rooting in cuttings of the flower crop, and that replaces the commercial rooting hormone. Thus, the biostimulants are an eco-friendly substitute to the high-cost chemical inputs used in the floriculture sector. The review aims to survey the various categories of biostimulants used for flower crops and their responses. The future of biostimulants in floriculture is very promising, making them an integral part of next-generation flower production.

KEYWORDS: Biostimulants; Floriculture; Nutrient-use Efficiency; Stress tolerance

1. INTRODUCTION

In the current era, the agricultural and horticultural sector has started moving towards sustainable production to reduce environmental impacts and improve human health. Various technological advancements were developed for promoting eco- friendly practices, thereby reducing the use of chemical inputs. The floriculture industry is now focusing on producing healthy and eco-friendly products, meeting the consumer's demand. Plant biostimulants contain natural constituents that promote plant growth by physiological and molecular processes, effective even when applied in small quantities. It is considered a promising innovation that increases the yield, quality, and productivity of crops (1, 2). Still, there is no clear definition of biostimulants, and the EU generally classifies them as fertilizers or pesticides. The function is to accelerate the crop cycle, increase the number of flowers, and improve the drought tolerance of turfgrass. Several compounds are said to be biostimulants, such as humic substances, Complex Organic materials, Seaweed extracts, chitosan derivatives, and protein hydrolase. Among these, the humic and microbial- based substance shows hormone-like activity in plants(3). Biostimulants are said to increase nutrient-use efficiency and mitigate environmental stress in crops. The humic substance improves soil fertility by enhancing the structure, and through biostimulatory action, it protects the plant from salinity and drought. The seaweed extract has plant growth-promoting benefits and enhances the shelf life of flowers. Plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi enhance root growth, thereby improving nutrient uptake and photosynthetic efficiency of plants. The protein hydrolase and amino acids were environmentally friendly, which enhances the mobility of micronutrients and promotes growth in horticultural crops(1, 4). Chitosan has antioxidant properties that increase the plant's tolerance towards abiotic stress and prevent yield loss. In general, biostimulants are widely used on high-value greenhouse-grown ornamentals and vegetables to enhance productivity sustainably (5). The application of biostimulants like humic substances, seaweed extract, and protein hydrolase promotes vegetative growth in flower crops by enhancing root growth and nutrient acquisition efficiency. They have a significant impact on flower yield and quality, thereby improving their commercial value in the market (2). The volatile compounds and essential oil content also increased in the flower crop when a biostimulant was used. (6). Existing research has shown that biostimulants play an important role in increasing flower crop productivity as well as quality.

In the global market, biostimulants account for 25%- 30%, and a major factor influencing the growth was the diverse availability of products and environmental concerns. Usage of biostimulants was gaining interest in sustainable horticulture, thereby helping in reducing the use of chemical fertilizers in commercial flower crops (4, 7). This review provides an overview of biostimulants, evolution, classification, functional response and their impact on the flower crops. Additionally, it highlights the performance of the flower crop under stress conditions.

2. Biostimulants: Concept and Regulatory Perspective

2.1 Evolution of the Biostimulant Concept

During the earlier period, they were broadly referred to as "biogenic stimulants" and "metabolic enhancers". Later, scientists and industry began to exclude classical nutrients and pesticides, emphasising small doses that enhance growth and stress tolerance, then calling them biostimulants. These are materials other than fertilizers that promote plant growth when applied in minute quantities and are also known as metabolic enhancers. They promote plant growth besides improving yield and quality. In the present, they were promoting the more effective and efficient use of mineral fertilizers and synthetic chemicals, 'the growing frequency of unfavourable environmental conditions for crop growth and productivity, and the growing availability of new biostimulant products that address specific agronomic and horticultural needs are some of the factors driving the rapid growth of biostimulants in the global market'(1, 3).

2.2 Regulatory Status of Biostimulants

According to 2019/1009 regulation of EU Fertilising products, Plant biostimulant is a "fertilizing product as its function to enhance plant nutrition processes without regard to the nutrient content, intending to improve one or more characteristics of the plant or its rhizosphere: (a) efficiency of nutrient use, (b) resilience to abiotic stress, (c) quality traits, or (d) accessibility of limited nutrients in the soil or rhizosphere."

The 2018 Farm Bill (Agriculture Improvement Act of 2018) directed the US Department of Agriculture (USDA) to analyze regulatory concerns regarding biostimulants (The Government of the United States, 2018). Although an official definition is still pending, the bill provided an initial description of biostimulants as 'a substance or micro-organism that, when utilized on seeds, plants, or the rhizosphere, promotes natural processes to improve or support nutrient uptake, nutrient efficiency, tolerance to abiotic stresses, or crop quality and yield' (The Government of the United States, 2018). In the United States, biostimulants are currently regulated based on the claims made for the products. They are classified as either pesticides or fertilisers under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), although there is no formal definition (Biological Products Industry Alliance, 2022; United States Department of Agriculture, USDA, 2019).

The Fertiliser (control) order 1985, by the order of March 2006, Biostimulants are considered as a type of 'biofertilizers', and defined as 'any product containing a carrier (solid or liquid) with living microorganisms that are agriculturally useful in terms of nitrogen fixation, phosphorus solubilisation or nutrient mobilisation to increase soil and/or crop productivity.' (8, 9)

3. Classification of Biostimulants Used in Flower Crops

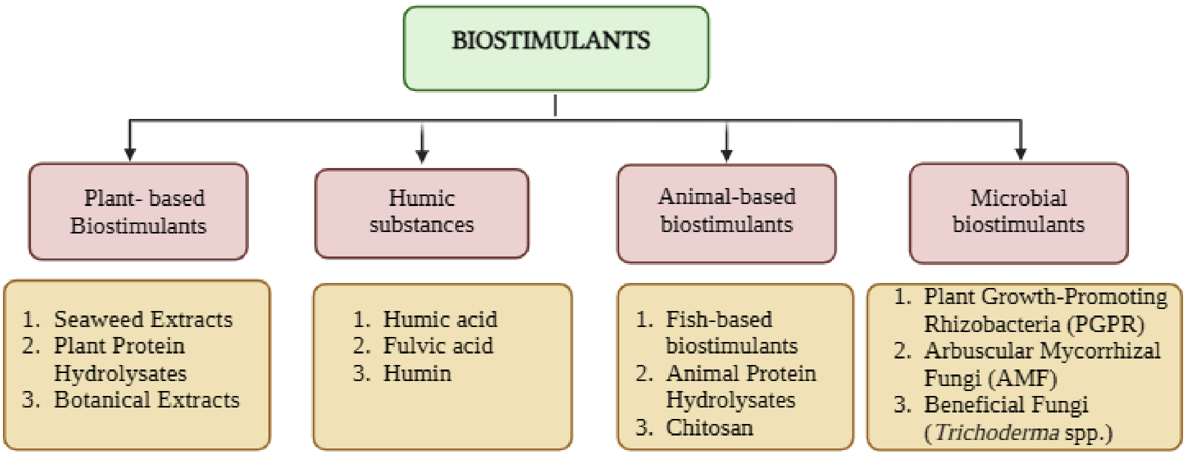

Figure 1: Classification of biostimulants based on their source of origin

3.1 Plant-Based Biostimulants

Biostimulants are most widely used in crop production, and their use in sustainable agriculture is growing. Plant-based biostimulants are products derived from plants, such as seaweeds and terrestrial plants, that are applied in trace amounts to support natural processes in crops, increasing yield/quality, stress tolerance, and nutrient use efficiency, which is better than fertilizers and pesticides (1). Fig 1 describes the broad classification of biostimulants. The application of plant-based biostimulants can improve the quality of produce and boost plant performance by initiating new root growth and increasing the antioxidant content, as well as leaf pigments(10).

3.1.1 Seaweed Extracts

A vital part of marine coastal ecosystems is seaweeds. These include multicellular, macroscopic marine algae that frequently live in coastal areas of the world's oceans when adequate substrates are available. According to estimates, there are over 9,000 species of macroalgae, which can be broadly divided into three groups according to their pigmentation (e.g., Phaeophyta, Rhodophyta, and Chlorophyta; or the brown, red, and green algae, respectively) (11). Seaweed extracts have consistently been demonstrated to contribute to plant growth promotion, enhanced yields, and plant tolerance to abiotic and biotic stresses (12). Both naturally occurring and commercially produced marine algae species contain molecules of chemicals that resemble cytokinin, auxin, and gibberellin (13)

The seaweed extract products on the market are mostly derived from brown seaweeds like *Ascophyllum nodosum, Ecklonia maxima, and Macrocystis pyrifera*. They contain pure substances like polysaccharides (laminarin, alginates, and carrageenans) and their breakdown products. Sterols, micro and macronutrients, n-containing substances like betaines, and hormones are some of the other things that contribute to plant growth (2).

3.1.2 Plant Protein Hydrolysates

Protein hydrolysates and amino acids derived from plants, called as plant protein-based biostimulants, are readily available because of the abundance of raw materials and their affordable cost. (14). Chemical and enzymatic protein hydrolysis of agro-industrial byproducts generally produces plant-based amino acid and peptide mixtures. Betaines, non-protein amino acids, and polyamines are some other nitrogenous molecules present in PHs that have beneficial effects on plant growth (2).

Amino acid-based biostimulants are derived from plant proteins such as soybean, legumes, corn, algae, alfalfa, seeds, etc., through chemical synthesis. Amino acids are also important in the agriculture industry, as they are environmentally friendly, which decreases the application of inorganic fertilizers(14). Protein hydrolysates (PHs) manufactured through partial hydrolysis from plant protein sources are a category of plant biostimulants defined as 'mixtures of polypeptides, oligopeptides and amino acids'. Besides peptides and amino acids, PHs can contain carbohydrates, minerals, phenols, and phytohormones, as well as some other organic substances, whose concentration is usually negligible (11, 15). This can be applied as foliar, root application, mixed with nutrient solution, and even seed priming; there was no phytotoxicity and growth depression was observed on foliar applications.(11). Protein hydrolysates (PHs) are gaining attention in open-field agriculture and protected cultivation(16).

3.1.3 Plant Extracts

Plant or Botanical extracts are easily derived from physical methods, like pressure-based, thermal and microwave, and chemical methods using organic solvents, alkali and acids. The extract contains bioactive compounds of plants, which improve the plant's resistance to pests and diseases as well as other abiotic factors, and it enhances the overall growth and productivity. To produce soluble plant metabolites, different extraction methods can be employed to effectively reduce the complexity of the plant tissues, thereby separating the bioactive substances from the insoluble material using an appropriate solvent. The prepared extracts can be applied through foliar spray, soil application, or a combination of the two approaches. Fertilization, drenching, and/or drip application systems can be used for application of extract into the soil or growth media(17, 18). Moringa and aloe vera extract have been reported to be used in orchids and gladiolus(19, 20).

3.2 Humic substances

In soil, the major part of organic matter is humic substance. It is obtained through decomposition of microbial processes and chemical degradation of plant, animal, and microbial residues that are present in the soil. These Humic substances include humic acids (HA), which are alkali-soluble but acid-insoluble, fulvic acids (FA), soluble in both acid and alkali, and humin, insoluble in both acid and alkali. In soil, 60% of the organic matter is composed of humic substances (21). This enhances soil fertility by changing its physical, physicochemical, chemical, and biological characteristics(22) .HS helps in mineralization of organic matter, nutrient solubilization and thereby promotes plant growth(23).

3.3 Animal-Based Biostimulants

Animal-based biostimulants are derived from the processing of animal by-products, such as epithelial tissue, collagen, leather, and leftover fish waste, which are rich in animal protein. These combinations and products available on the market contain bioactive compounds that help in plant development and strengthen resilience to abiotic stress.(24, 25).

3.3.1 Fish-based biostimulants

Fish protein hydrolysates are rich in free amino acids and peptides. It is easily digested and hydrolysed by enzymatic, alkaline, or acidic methods. These have antioxidant and antimicrobial properties that improve the plant's health(26, 27).

Protein hydrolysates from fish and fish by-products are obtained by various methods, such as autolysis, thermal hydrolysis, chemical hydrolysis, and enzymatic hydrolysis. Generally, FPH are rich in Glutamic acid, which improves plant growth and yield.(28, 29)

3.3.2 Animal Protein Hydrolysates

Animal protein hydrolase accounts for 90% of the PH market. These products are obtained through chemical hydrolysis of proteins of animal origin, such as collagen from leather by-products, in Europe, India, and China. Enzymatically produced PHs from plant biomass are less common because it has been recently introduced to the biostimulant market. (4).

Under Commission Implementing Regulation (EU) No. 354/2014, the use of animal-derived protein hydrolysates on the edible parts of organic crops has been banned, and the restriction remains in force until 2026(3)

3.3.3 Chitosan

Chitosan is prepared by eliminating 80% of the acetyl groups from N-acetyl-d glucosamine residues of chitin. It is a monomeric unit: $C_3 H_{11} NO_4$, or 2-amino-2-deoxy-β-D glucopyranose, mostly obtained by deacetylation from chitin and is made up of glucosamine units. (30). Chitosan can also be prepared by extracting from mould cell walls. Chitin and chitosan are biodegradable and non-toxic natural compounds (31). Because of their unique properties, chitosan-based products can be used as plant biostimulants in a variety of industries, including agriculture. Polycationic chitosan has numerous application in agriculture and horticulture, e.g., as an elicitor, soil modifier, films and fungicide(32).

3.4 Microbial Biostimulants

Microbial biostimulants are available commercially in markets that include the mycorrhizal or non-mycorrhizal fungi and bacteria group of microorganisms, which colonize the rhizosphere and improve soil aggregation, soil fertility and promote plant growth.(33) Bacteria, yeasts, and filamentous fungi that have been isolated from soil, plants, and other organic materials make up the majority of these biostimulants. It can be applied to the seeds as well as the soil, which have direct or indirect effects on crop productivity. Flower species such as *Petunia hybrida* and *Chrysanthemum morifolium* have shown improved flowering performance followed by the application of microbial biostimulants. However, limited information is available on the response of bedding flowers like *Tagetes patula*. The collective application of microbial biostimulants and organic fertilizers may promote the growth of ornamental flowers(34)

3.4.1 Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria (PGPR), are known to strengthen soil structures, deliver nutrients, and generate hormones, which help with plant development. It also improves the plant response to biotic and abiotic stress. Bacterial-inoculated biostimulants include species such as *Azotobacter spp., Acinetobacter spp., Pseudomonas spp., Rhodococcus spp.,* and *Bacillus spp*. Some flower crops, such as tuberose and rose, have shown positive responses to microbial inoculation and plant growth–promoting inputs.(35, 36)**.**

3.4.2 Arbuscular Mycorrhizal Fungi (AMF)

Arbuscule-Forming Mycorrhiza (AMF) forms a symbiotic association with fungal hyphae that penetrate into cortical cells of plants and develop a branched structure called arbuscules, a common form of endomycorrhiza associated with agricultural and horticultural crops. These were applied to plants and should be classified as biostimulants since they increase nutrient absorption, stress tolerance, and crop yield(3). Due to their biotrophic nature, there is a technical difficulty in propagating AMF on a huge scale, and this is a major limitation for their restricted availability. Arbuscular mycorrhizal fungi are reported to improve the fresh and dry shoot weight of Zinnia(36).

3.4.3 Beneficial Fungi (*Trichoderma* spp.)

In horticulture, the biocontrol fungus *Trichoderma* has gained significance as a microbial plant biostimulant. The capacity of certain *Trichoderma* microbial strains acts as an active ingredient in biopesticides used in agricultural management techniques, such as Integrated Pest Management (IPM), because of their ability to protect against phytopathogens and help in the development of crops with resistance (37). *Trichoderma*-based biostimulants applied to *Zinnia spp*. and *Tagetes patula L*. improved plant growth and flower quality by enhancing vegetative growth and overall plant vigour(36).

4. Functional Responses of Flower Crops to Biostimulant Application

4.1 Root System Development and Soil Microbe Dynamics

Biostimulant application plays a crucial role in improving root system architecture in flower crops by stimulating primary root elongation, lateral root development, and root hair density. These changes enhance the effective root surface area, allowing better soil exploration and anchorage. The development of roots would significantly improve plant productivity and help alleviate stressful conditions by maximising water uptake and nutrient-use efficiency(15) . According to some authors, even the soy protein hydrolase can affect the makeup and activity of soil microorganisms, which may therefore contribute to the beneficial effects seen in crops. Positive effects of PHs on root growth and development have been reported in Lily (38).HA treatments stimulate root growth, and the application of 40 mg/L HA severely affected the root surface area, also increasing the index by 120% compared to the control in Gerbera (39). Physiological responses such as nutrient mobilisation, distribution, and the development of a vigorous root system are observed in plants with the foliar application of seaweed extract. The small amount of phytohormones present in the seaweed extracts promotes the plant system's stimulatory processes, improves the rooting architecture of plants, as reported in(40)

The development of plant root systems treated with seaweed extracts was also observed in vegetatively propagated flower crops. For instance, cuttings of marigold treated with an extract from *E. maxima* increased the root density(40). *Ascophyllum nodosum*, brown seaweed extract, increases the germination capacity of seeds in *Tagetes erecta L*.(12).The root length and total root surface area were increased in petunia on the foliar application of the animal-derived PH biostimulant (41).The collective application of AMF and PGPR increased the root length, dry and fresh weight of the root in Petunia (42).Thus, all kinds of biostimulants applied to the flower crop will improve the root architecture.

4.2 Biostimulant-Induced Nutrient Mobilization and Assimilation

Biostimulants enhance nutrient acquisition efficiency by increasing the availability of nutrients in the rhizosphere and enhancing the plant's ability to absorb and utilize nutrients. The improvement of nutritional status and resource utilization observed when there is an increase in nutrient absorption and assimilation by increasing root biomass, density, and lateral root branching (43). Humic substances are attributed to several mechanisms, including improved soil structure, increased nutrient availability, and nitrate assimilation(1). In some crops, biostimulants raise the levels of soluble sugars, and thus, it is an important energy source that is also used for the synthesis of some primary metabolites(44). Humic substances stimulated H1 adenosine triphosphatase activity and the activation of ion transporters; upregulation of glutamine synthetase and glutamine synthase activities leads to higher nitrate uptake and assimilation (45, 46). Foliar application of humic acid helps in the quick absorption of nutrients, enhancing enzymatic activity to support cellular processes and promoting root growth in Gladiolus and chrysanthemum. (47) (48).

Researchers have noted the value of seaweed extract in enhancing plant growth by increasing the uptake of nutrients from the soil (49). Physiological responses due to seaweed extract spray include improved nutrient mobilization, partitioning, and the development of a vigorous root system, thereby enhancing the utilization of soil nutrients in *Jasminum sambac* (13). Increased uptake of nutrients and osmotic uptake of water under the influence of biostimulants causes better plant height and internodal length in chrysanthemum under greenhouse conditions(50).Amino acids have indirect impacts on plant growth and development because they are easily absorbed, transported, and used by plants as a source of carbon and nitrogen. It increases microbial activity and improves the structure and fertility of the soil (22).The NPK content is increased in the leaf due to enhanced uptake of nutrients on the application of amino acid-based biostimulants, reported in Gerbera and tuberose, and micronutrients Cu, Mn and Zn are additionally increased in leaves of *Zinnia sp.,* (51, 52).

4.3 Enhancement of Physiological Performance under Stress Conditions

A biostimulant not only promotes growth but also helps plants to function well under stress conditions by developing tolerance and strengthening the immune system. These biostimulants regulate and stimulate gene expression, enzyme activity, metabolic changes, hormone influence, and, through this stress response, nutrient intake is managed in plants. Under abiotic stress, plants respond through different biological and physiological processes, such as ROS scavenging mechanisms, maintaining membrane integrity, Osmo protection, stomatal behaviour and transpiration control, xylem conductivity, rhizosphere nutrient availability, and metal chelation (5). As a defence response to biotic stresses, it develops physical and chemical barriers, such as structural protection by cutin, waxes, and cell walls, and utilises defence components, including antimicrobial enzymes and secondary metabolites, to prevent invasion by various pathogens. Biostimulants are said to induce ROS-detoxifying enzymes and non-enzymatic antioxidant compounds under abiotic stress. In addition, microbial-based biostimulants enhance the plant defence mechanism that develops tolerance against biotic stress(36). Seaweeds are said to improve tolerance against salinity and drought(5, 53). The application of SWEs stimulates the antioxidant enzymes and accelerates the activities of several molecules (e.g, α-tocopherol, ascorbate, and β-carotene(54). It is delivered that seaweed extract helps in maintaining the membrane integrity in cells and preventing electrolyte leakage in petunia under water stress (55). In Hydrangea, *A. nodosum (*seaweed extract) reduces stem shrinkage under drought(56).

Under stressful conditions, these humic compounds can control plant growth and can scavenge free radicals by promoting antioxidant activity (7). The application of HA resulted in a positive effect and enhanced growth at all levels of salt stress, thereby acting as a growth enhancer. HA at 1000 mg/L showed the highest resistance to high salinity (4000 mg/L) and improved the growth of geranium (57). Recent studies on *Calendula officinalis* have demonstrated that the FA treatment under salinity conditions significantly increased chlorophyll content, antioxidant activity, and ionic balance(58). Chitosan increases the growth regulators such as indole acetic acid, gibberellin, and abscisic acid, thereby reducing oxidative stress and increasing crop yields, and thus it helps the plant to survive under both biotic and abiotic stress conditions. The *Calendula officinalis* on the application of the optimum dose of Chitosan (7.5 mg L^{-1}) considerably increased leaf water status, membrane stability, pigments, gas exchange, and stomata characteristics, which are important traits to improve the resistance to water stress (59). The effect of salt stress on Zinnia is reduced through the application of chitosan, and it seems to increase the K^+ content, K^+/Na^+ ratio, and also the activity of antioxidant enzymes(60). Similarly, the chitosan increased the ion composition that aided in osmotic adjustment, and it showed increased expression of antioxidant-related genes in Chrysanthemum(61) Fig 2 shows the effect and response in plants by chitosan. The arbuscular mycorrhizal fungi and PGPR have a positive impact on the growth and development of plants under drought conditions. The application of mycorrhizae and rhizobacteria in petunia helps mitigate drought stress and improves root parameters and flowering characters (42). Also, in *Calendula officinalis,* the bacterial inoculation with *Azotobacter chroococcum*, *Bacillus megaterium*, and *Pseudomonas fluorescens* effectively mitigates salt stress and promotes the growth and flowering(36). These biostimulants were effective and sustainable for moderating stress in plants.

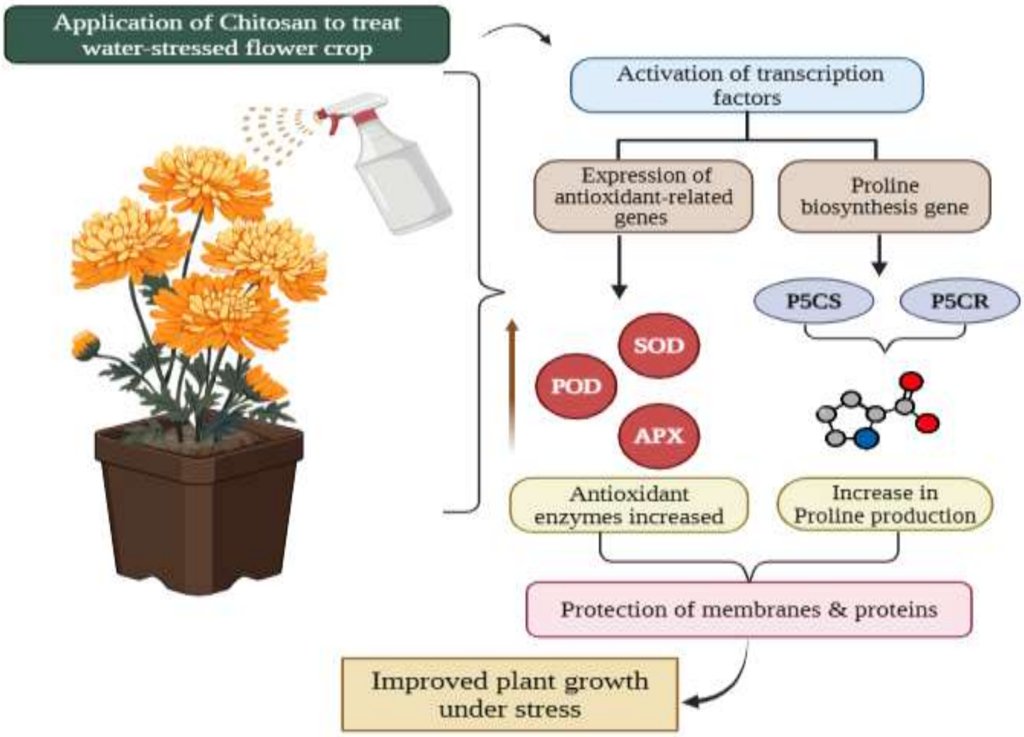

Figure:2 Chitosan-mediated regulation of stress response in flower crops

5. Impact of Biostimulants on Vegetative Growth of Flower Crops

5.1 Role in the Propagation of Flower Crops

Seed propagation, tissue culture and vegetative cuttings are the important commercial propagation methods of flower crops. Among these, vegetative propagation is highly practiced and the biostimulants improve the propagation efficiency by exhibiting hormone-like activities which increase the root differentiation, which reduces the loss from insufficient rooting (62). The *Ascophylum nodosum* enriched biostimulant 'rootnectar' reduce time taken for the formation of adventitious roots and root branching in cuttings of chrysanthemum compared to the control. (63). Rose cuttings soaked in moringa leaf extract shows highest rooting percentage, root length and root number compared to other biostimulants(64). A pansy (*Viola tricolor* var. hortensis) seedling treated with a biostimulant developed more roots compared to the control, as reported in(65). In addition, Chitosan and coconut water have a positive impact on the orchid '*Cattleya maxima'* (19)**.**

5.2 Plant Growth and Biomass Accumulation

They play a crucial role in promoting plant growth through both direct pathways (plant metabolism) and indirect pathways (soil fertility). It also enhances the quality of the crops by increasing the yield through nutrient uptake. Seaweed contains macro- and micronutrients, as well as small quantities of phytohormones that promote plant growth. In a pot study of gerbera, the application of 3.0 (g/L) gives the highest value for the measured plant parameter, number of leaves(66). The biomass, plant height and number of leaves were increased in the following crops: *J.sambac*, *Petunia hybrida*, China aster and Dahlia through the application of seaweed extract(67, 68). Humic and fulvic acids improve plant metabolism by altering biochemical processes and increasing nutrient uptake. Application of humic and fulvic acids improved plant height and biomass in Gladiolus, and increased plant spread in Chrysanthemum(69, 70). The protein hydrolase increases the biomass by stimulating gibberellin-like activity in plants. The studies reported that quality and yield are increased in Gerbera and *Freezia hybrida* by Plant protein hydrolase application(71, 72). Similarly, the biomass is increased due to enhanced photosynthetic capacity in *Petunia hybrida* and Chrysanthemum by the application of animal–based PH(41, 73). (Table 1) gives the overall effect on vegetative growth of flower crops by biostimulants. Collectively, the application of the biostimulant improves the biomass, and it creates a larger influence on vegetative growth**.**

5.3 Leaf Morphology and Chlorophyll Content

Biostimulants have been shown to positively influence leaf morphology and chlorophyll content in various flower crops, enhancing growth and productivity through chlorophyll biosynthesis and phytohormone content in flower crops(74). The total chlorophyll content is increased in Chrysanthemum(70), Gladiolus(69) and tuberose(75) under humic acid application. The seaweed extract and protein hydrolase increase the leaf area by supplementing micronutrients to the plant. China aster, Lilium, and Gladiolus show the highest leaf area with the foliar application of seaweed extract(67, 76, 77). The high dose of animal-derived PH on petunia yields the highest leaf area among the treatments (41). It is also reported that the chlorophyll content is increased by the application of seaweed extract of 1500 ppm in *Calendula officinalis* and Dahlia(68, 78). External Chitosan application improved chlorophyll and carotenoid concentrations by increasing enzymatic activity (CAT, GPX, SO, which helps to limit lipid peroxidation under water stress conditions. Chitosan application also increases the Chlorophyll a, b, and carotenoid content in Gomphrena, Gladiolus, and tuberose, as measured by the SPAD index (79-81). The biostimulant improves chlorophyll synthesis, thereby increasing light absorption and enhancing photosynthesis in plants.

Table 1: Effect of Biostimulants on Vegetative Growth of Flower Crop

Crop	**Biostimulants**	**Dose/concentration**	**Key response**	**Reference**
Rosa chinensis L.	IIHR Arka microbial consortium + Humic acid	Foliar application HA @ 3 ml/litre, AMC @ 20 g/litre	Increased plant height, spread, and number of leaves	(82)
Gerbera jamesonii	Mixture of amino acids	Foliar application of 100 ppm	Increased total leaf area, number of leaves, and chlorophyll	(51)
	Seaweed extract	Foliar application of 1g/ litre	Enhanced greenness and chlorophyll content of the leaf	(83)
Tuberose	Humic acid	Foliar application of 150 mg /litre	Highest stem length, spike length and chlorophyll content (52 mg g-1) compared to the control	(75)
	Chitosan	Foliar application of 60 ppm	Increased plant height and number of leaves	(79)
Gladiolus	Humic acid	Applied as split doses, 4 kg / Hectare	The maximum number of leaves and leaf area recorded	(69)
Lilium asiatica	Seaweed extract (Biovita)	Foliar application of 2ml/litre	Increased plant height, stem diameter and Number of leaves	(76)
Chrysanthemum	Azospirillum and PSB	5kg/ hectare along with the application of NPK	Maximum plant height, number of leaves, branches and leaf area observed	(84)
Petunia	Animal based PH	Foliar application of 0.2g/ litre	Maximum root length, total root surface and number of leaves obtained	(41)
	Seaweed extract	Foliar application of 6ml /litre	The highest mean values of plant height, number of branches per plant, and leaf area were observed	(55)
China aster	Seaweed extract and microbial biostimulant	Foliar application of 0.5% SW with PSB @ 200 g + KSB @ 200 g	Increased plant height, number of branches, leaf area and number of leaves	(67)
Calendula officinalis L.	Chitosan	Foliar application of 7.5 mg /litre	Increased the number of leaves and leaf area	(59)

6. Influence of Biostimulants on Flower Yield and Quality Attributes

There is a great need for environmentally sustainable farming methods due to the growing demand for flowers. To increase the growth, yield, and quality of commercially significant flower crops, sustainable crop production techniques that utilise organic fertilisers and natural biostimulants are being promoted. The biostimulants like seaweed extract, humic substance, and protein hydrolase are reported to improve the yield and quality traits in flowers. The number of flowers and the diameter of flowers are greatly influenced by seaweed extract application in the China aster (67), *Tagetes patula(85),* Gladiolus(77), Gerbera (66), and Lilium(76). Protein hydrolase and humic substances have positive effects on flower yield and size. The studies show that the number of flowers is increased in tuberose(75) , Gladiolus (69) and Chrysanthemum(70) through the foliar application of humic substances. The plant-based protein hydrolase increased the diameter of the flower in the carnation (86) and gerbera (51). The AMF regulate flower development and promotes early flowering by altering the endogenous hormones ABA, GA3, IAA in *Antirrhinum majus* L. (87). Fig 3PGPR, such as *Azospirillum, Azotobacter*, PSM and KSB, influence flowering and increase the yield in *Rosa hybrida* cv. Gladiator(88), Chrysanthemum by *Azospirillum*(89), and *Gladiolus grandiflorus* cv. Nova Lux performs well when it is supplemented with vermicompost(90). Chitosan induces early flowering in Rose(91), increases the inflorescence length in gladiolus(80) and improves the flowering parameters of tuberose(79).(Table 2) summarizes the influence of different biostimulants on flower characters.

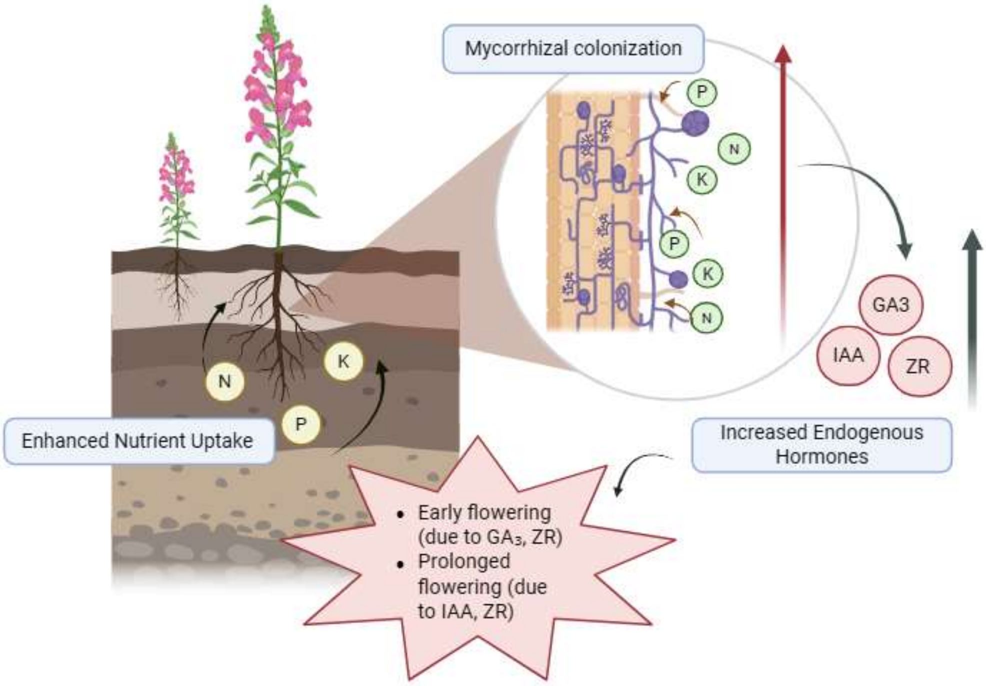

Figure 3: Role of microbial biostimulants in nutrient uptake, phytohormonal regulation and flowering

Humic substance and protein hydrolase are reported to improve photosynthetic pigments, carbohydrate accumulation, and flower growth characteristics, which increases overall flower vitality, flower quality and prolongs vase life. The biostimulant use through various strategies that enhance water uptake, reduce microbial growth, and boost antioxidant production in flowers. The humic substances are reported to enhance the vase life of cut flowers and extend the longevity of Gladiolus cv Priscilla by 3.2 days (69), Chrysanthemum by 7.8 days(70), and Gerbera by up to 3.6 days compared to the control. The vase life was also extended by applying seaweed extract and protein hydrolysate; Lilium vase life was recorded up to 12.60 days, and chrysanthemum showed improved longevity. However, it was found that plant-based protein hydrolysates increased the flower's longevity, while animal-based protein hydrolysates decreased vase life(73, 76). Also, the vase life and membrane stability index % are enhanced in the gladiolus cv. White Prosperity by moringa leaf extract(20). The above studies state that sustainable practices can improve flower yield, quality, and vase life and act as a potential alternative to conventional practices.

Table 2: Effect of biostimulants on flower yield and quality

Crop	Biostimulants	Dose/Concentration	Key response	Reference
Carnation	Amino acids and peptides (Hicure)	Applied as a soil drench at 3 litres/hectare	Slight increase in flower head diameter and yield, but vase life is reduced	(86)
	Moringa and mint leaf extract	Foliar application of 0.03%	Reduce the salinity stress and improve flower weight	(92)
Gerbera	Mixture of amino acids	Foliar application of 100 ppm	Increased flower head diameter, number of flowers and stem length	(51)
Gladiolus	Humic acid	Applied as split doses, 4 kg / Hectare	The number of florets /spike, Spike length and vase life were enhanced	(69)
	Chitosan	Foliar application of 0.4%	Increase in the number of inflorescence shoots and the length of the inflorescence	(80)
Dendrobium orchid (Sonia-17)	Seaweed extract (*Ascophyllum nodosum*)	Foliar application of 1.5%	Enhanced the spike initiation	(93)
Chrysanthemum	Humic acid	Foliar application of 0.2 %	Maximum number of flowers per plant and yield; shelf life extended	(94)

Tuberose	Chitosan	Foliar application of 60 ppm	Increased number of florets/spikes, spike length and rachis length	(79)
French marigold	Seaweed extract	Foliar application of 6.0 ml/litre	Maximum number of flowers; increased carotenoid content, and essential oil	(85)
Roses var. 'Local'	Seaweed extract (*Padina antrillarum*)	Foliar application of 20 %	Maximum number of flowers	(95)
Gomphrena globosa	Humic acid	Foliar application of 700mg/litre	Increased number of inflorescences, inflorescence diameter and inflorescence height	(81)

7. Effect of Biostimulants on Floral Fragrance and Volatile Compounds

Floral scents are a combination of terpenoids, phenylpropanoids/benzenoids, fatty acids, and amino acids. The biosynthesis of these volatiles involves complex pathways regulated by genes, transcription factors and environmental factors. Moreover, biostimulants such as Humic substance, seaweed extract and amino acids significantly influence secondary metabolism, which improves essential oil content in medicinal and flower plants(6, 96). The humic acid up-regulated a variety of differentially expressed genes related to monoterpene biosynthesis pathways, which are responsible for the smell of roses. This process of activating the metabolic pathway increases the activity of enzymes and encourages the production of volatile compounds associated with the smell of *Rosa rugosa* (97). Seaweed extract and chitosan have been reported to increase the level of volatile oil in *Pelargonium graveolens* by modulating the key biosynthetic genes and secondary metabolic pathways involved in the synthesis of volatile organic compounds (VOC)(98). Overall, from the studies, we can conclude that biostimulants regulate secondary metabolism, which enhances the fragrance of flowers.

CONCLUSIONS AND FUTURE PROSPECTS

In sustainable cultivation, biostimulants have played a vital role in improving the productivity of commercial crops. A wide range of categories and products is available in the market, that were shown a positive impact on flower crops. The application of biostimulants reduces the overuse of fertilisers and, in addition to improving yield, it enhances the tolerance of flowers to biotic and abiotic stress. Most research studies report the beneficial effects of biostimulants on flower crops; only limited studies have focused on their mechanisms of action and associated biochemical changes. Furthermore, biostimulants such as humic acid, seaweed extracts, protein hydrolysates, chitosan, and microbial inoculants differ in their composition, resulting in varied responses in flower crops across these categories. Since these biostimulants are chemically complex and heterogeneous, their optimal usage for crops is not clearly defined. The major limitations were inconsistent responses among plants and a lack of awareness among the farmers.

Future research should focus on the mechanisms of biostimulants using omics tools to gain deeper insights into their effects on flowering and quality traits. Crop-specific guidelines on the application of biostimulants, including application methods, dosages, etc., would be beneficial for using the product effectively in the field. Moreover, the use of multi-component formulated products can help to apply multiple biostimulants at once. Further studies need to concentrate on long-term field trials in diverse agro-climatic conditions to validate their consistency and effectiveness. However, biostimulants are gaining attention among farmers and researchers with continuous advancements in formulations and applications.

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Authors contributions

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Conflict of interest

The authors declare that they have no conflict of interest.

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