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Lamabam Juliana Devi
Research Scholar, Department of
Horticulture, School of
Agriculture, Lovely Professional
University, Phagwara, Punjab,
India

Vandana Thakur
Department of Horticulture,
School of Agriculture, Lovely
Professional University, Phagwara,
Punjab, India

Effect of Biostimulants on horticultural crop aspects, biotic and abiotic stress management

Lamabam Juliana Devi and Vandana Thakur

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Abstract

Biotic and abiotic stresses have a significant impact on plant growth, development, and quality of production. The effectiveness of biostimulants in mitigating stressful conditions is contingent upon various aspects, including the time of their application and their manner of action. Biostimulants are natural or synthetic substances applied to plants, seeds, and soil to enhance nutritional efficiency and improve tolerance to abiotic stress and crop quality traits, regardless of their nutrient content. Beyond basic nourishment, algal extracts, protein hydrolysates, humic substances, microorganisms, and inorganic chemical combinations frequently improve growth and stress tolerance. In this review, we review and summarise the literature on the use of biostimulants on vegetable crops, with an emphasis on their application to counteract the most prevalent environmental stresses.

Keywords: Humic substances, stress, mitigation, quality traits

Introduction

Horticultural crop yield and production have been greatly impacted by both human activity and the changing global environment (Varshney). Plants undergo numerous stressful events throughout their life cycle, from seed germination to maturity. Depending on the type of trigger element, these stresses are usually divided into two categories: biotic and abiotic stresses. Biotic stresses are caused by other living things that affect the growth and productivity of plants, such as weeds, bacteria, fungi, and insects. In contrast, abiotic stresses are due to the climatic, edaphic, and physiographic elements of the environment are factors that restrict plant growth and survival. Abiotic stress conditions include salinity, sodic alkalinity, drought, temperature swings, and exposure to heavy metals, among other environmental limitations (Lal *et al.*, 2022)^[31]. It has been established that abiotic stress affects a plant's capacity to develop and yield by inducing physiological, morphological, anatomical, biochemical, and molecular alterations (Rao *et al.*, 2016)^[38]. The processes of seed germination, growth, reproduction (including seed development, flowering, and fruiting) and eventual decline in horticulture crops are impacted by both biotic and abiotic stress. Therefore, to determine the effects of abiotic stresses and identify potential resistance mechanisms and ameliorating strategies, it is essential to study the physiological, biochemical, and molecular reactions of horticulture crops.

To mitigate the negative effects of biotic and abiotic stresses on agricultural plants, horticulture crops have undergone a variety of interventions (Williamson *et al.*, 2002; Sonmez *et al.*, 2009). These include substances that have been demonstrated to improve crop plant tolerance and ultimately result in enhanced yield in horticulture crops, such as hormones, biostimulant compounds, new chemicals, and microorganisms (Chen *et al.*; Jaiswal *et al.*). It is difficult to identify and continues to study the processes that biostimulants trigger in response to stressful situations. The effectiveness of biostimulants in mitigating stressful conditions is contingent upon various aspects, including the time of their application and their manner of action. Biostimulants can be applied at various times: before, during, or even after the stress that affects the culture. Stressful environments may cause the metabolic pathways that biostimulants activate to become more pronounced, which may aid plants in adapting and overcoming or delaying the most crucial times.

Corresponding Author:
Lamabam Juliana Devi
Research Scholar Department of
Horticulture, School of
Agriculture, Lovely Professional
University, Phagwara, Punjab,
India

1. Drought stress

One of the most common abiotic stresses that crops experience across various geographic regions is water stress (Mariani and Ferrante, 2017). Plant gas exchange is significantly impacted by drought stress, resulting in changes to transpiration and photosynthetic rates, which are directly related to yield. The noticeable indications of water stress include the leaves' turgor reduction and yellowing from chlorophyll deterioration. When plants are stressed by water, the amount of chlorophyll can be utilised as a useful indicator of a metabolic imbalance. When *Ascophyllum nodosum* was applied to broccoli (Kaluzewicz *et al.*, 2017) and spinach (Xu and Leskovar, 2015) ^[47], it improved gas exchange by reducing stomatal closure, which made the plants more resilient to water stress. Total chlorophyll content in tomato leaves was elevated by biostimulant treatments with a nodosum. After applying biostimulants, there was a decrease in water loss, wilting damages, and 3-carbon dialdehyde MDA. Water stress in plants can occasionally be attributed to bacterial infections that block xylem arteries, obstructing water flow. Tomato plant withering was postponed by treatments with *Azospirillum brasilense*, a strain isolated from arid conditions. Treated plants had a large area of xylem vessels, which led to a more effective transfer of water from the soil to the leaves

2. Salinity stress

As a result of osmotic stress brought on by salt, salinity is one of the primary abiotic stimuli that negatively affects plant development and metabolism. In saline conditions, sodium chloride (NaCl) is the more prevalent salt that is harmful in higher amounts (Viégas *et al.*, 2006). It is more common in coastal areas where crops are regularly irrigated with saline water (Colla *et al.*, 2010; Lucini *et al.*, 2015). Plant vitality and productivity are compromised by salt stress, which causes a decrease in plant development and damage at the cell level (Mariani and Ferrante, 2017). The length of exposure and the degree of stress both affect the crop. A common effect of salt conditions on plants is a notable decrease in fresh weight and chlorophyll content, which has also been noted in spinach (Kaya *et al.*, 2002) ^[29], beans (Taibi), and other crops (Yarsi *et al.*, 2017) ^[48]. The reduced uptake of nutrients from the soil caused by salt stress results in a nutrient imbalance, endangering the nutritional quality of horticultural crops. The pH of the soil solution also affects the solubility of micronutrients like Cu, Fe, Mn, Mo, and Zn, and in saline conditions, their availability is extremely low. The natural responses of plants to salinity are comparable to those seen in drought-stressed plants. Increases in proline, simple sugars, alcohols, abscisic acid, and antioxidant molecules that can prevent harm from free radical buildup can all be brought about by biostimulants. When biostimulants are applied, production losses are decreased and stress tolerance is raised. The species and the conditions of salt stress determine the number of applications. A plant-derived protein hydrolysate increased lettuce plants' capacity to withstand salinity, boosting production and dry weight (Lucini). When compared to the control, treated plants perform better and have a higher PS2 maximal quantum efficiency.

3. Cold stress

One of the most dangerous abiotic stresses that has a major impact on output is the decrease of the temperature or the return of cold temperatures in late spring (Ruzzi and Aroca, 2015). Low temperatures slow down physiological reactions and decrease plant metabolism. Cold stress causes reduced metabolism, which in turn causes photoinhibition known as the

suppression of photosystem II activity. The phospholipid layers in cell membranes become unstable due to the effects of cold. Low temperatures primarily damage cell membranes, which can result in growth retardation in less severe cases or death in more severe ones. The impact is severe, particularly for crops that flower early, but early tomato and melon transplants may also sustain substantial damage (Zang *et al.*, 2017; Ding *et al.*, 2017). Biostimulants derived from algae, amino acids, or microorganisms that can generate protection against low temperatures by accumulating cryoprotective compounds and/or activating membrane repair systems have demonstrated excellent results (Saegar *et al.*, 2020). To produce cold-protective substances, the biostimulant activity against cold stress often increases the accumulation of osmotic molecules by stimulating the biosynthetic pathways. Additionally, these biostimulants improve membrane thermostability, which minimises the chilling harm.

4. Heat stress

Agriculture is negatively impacted by global warming and rising temperature projections (Challinor *et al.*, 2014; Fahad *et al.*, 2017). Crops are susceptible from high temperatures due to changes in enzyme and cell membrane functions. Damage to membrane phospholipids specifically results from the lack of cellular compartmentalisation, which in turn causes metabolic problems. To improve thermoregulation in response to high temperatures, plants increase transpiration. The ideal temperature range for enzymatic activity and structural integrity is between 30°C and 45°C. Temperatures exceeding 60°C cause irreversible denaturation of the enzymes. Mung bean seedlings treated before being exposed to high temperatures demonstrated a decrease in oxidative stress and methylglyoxal content, a reactive substance that harms cells, after receiving an exogenous glutathione treatment against heat stress (Nahar *et al.* 2015). The biostimulants that promote tolerance in crops may work by boosting the ability of plants to absorb water as well as by encouraging the build-up of compounds that function as barriers to cell membranes.

Biostimulants

Today, feeding the world's growing population with fewer and more efficient inputs is our biggest issue (Bashir *et al.*, 2021). As a result, fertilisers have become essential to modern agriculture as they help in increasing crop production. Fertilisation increases efficiency and improves the yield and quality of the product. However, excessive use of chemical fertilisers depletes essential nutrients from the soil and minerals, hardens the soil, lowers soil fertility, pollutes the air, water, and soil, and creates environmental risks (Heena *et al.*, 2021). The primary objective is to meet the growing demand for food production using sustainable agriculture methods without compromising crop yield and producer revenue because of rising environmental contamination and the slow deterioration of cultivated soils (Russo and Berlyn, 1991). Organic farming helps in overcoming these problems by supporting and strengthening biological processes. Organic farming is a production system that uses biological fertilizers and pest control produced from animal or plant waste. Since chemical fertilisers and pesticides are used less frequently in organic agriculture (Pascual *et al.*, 2018), several plant amendments appropriate for such production are required. Biostimulants are employed nowadays since they are environmentally friendly and can produce food without harming the environment. Using biostimulants may be a viable solution in light of degraded

agricultural areas and climate change uncertainty. Biostimulants are natural or synthetic substances applied to plants, seeds, and soil to enhance nutritional efficiency and improve tolerance to abiotic stress and crop quality traits, regardless of its nutrient content. Biostimulants can act on the primary metabolism by increasing photosynthetic activity and derived compounds or they can stimulate the secondary metabolism by activating specific biosynthetic pathways (Bulgari *et al.*, 2019) ^[10]. It is nearly impossible to isolate and examine a single component, and the synergistic effects of several bioactive molecules are most likely responsible for a biostimulant's efficiency rather than a single substance (Rouphael and Colla). In fact, considering the challenges associated with characterising it, the composition of biostimulants continues to be the most crucial factor determining their efficiency. For all of these reasons, in 2016 a draft of a new fertiliser regulation was draughted, and the European Commission developed a proposal for a new regulatory framework. The European Parliament approved the modifications to the European Commission's proposal in October 2017, and on March 27, 2019, the legislative resolution about the plan was approved (Bulgari *et al.*, 2019) ^[10].

Classification of biostimulants and their effects on stresses

Over the years, different categorization of biostimulant on the basis of their origin, main component or mode of action has been put forward by many authors. In many countries outside the European Union, both kinds of information must be reported on the label to register these products (Yakhin). The current classification is based on source of raw material, even if this choice does not always provide the correct information about the biological activity of the product (Jardin, 2015). Thus, biostimulants are categorized into five major groups:

1. Seaweed extracts

Seaweeds are an extensive group of macroscopic and

multicellular algae which provide a good source of nutrients, bioactive compounds, fertilizers and organic matter. Red, green, and brown algae are examples of seaweeds, which provide up to 10% of marine productivity (Battacharyya *et al.*, 2015) ^[8]. Products made from seaweed have been shown to boost germination rates and substantially improve seedling vigour by increasing the size and density of the roots. The extracts from *Ascophyllum nodosum* are listed as the most frequently used (Spann *et al.*, 2011; Xu and Leskovar, 2015) ^[47]. Equally popular are *Solanum lycopersicum* L. (Hernández *et al.*, 2014), *Ecklonia maxima*, *Sargassum spp.* (Battacharyya *et al.*, 2015) ^[8], *Laminaria spp.*, *Durvillaea potatumum*, *Ulva lactuca*, *Caulerpa sertularioides*, *Padina gymnospora*, *Sargassum liebmannii*, and *Sargassum johnstonii* (Kumari *et al.*, 2011; Xu and Leskovar, 2015) ^[47]. They are applied as foliar spray and can enhance plant growth, abiotic stress tolerance, photosynthetic activity, and resistance to fungi, bacteria and viruses, improving the yield and productivity of several crops.

The seaweed extracts' bioactive components improve plants' ability to withstand abiotic stresses. It has been demonstrated that spraying extracts increases plants' resistance to freezing temperature stress (Mancuso *et al.*, 2006) ^[33]. Specifically, the administration of an *A. nodosum* extract formulation improved grape freezing resistance by lowering the leaves' osmotic potential, an important indicator of osmotic tolerance.

After nine days of seaweed extract treatment, the average osmotic potential of the treated plants was -1.57 MPa, compared to -1.51 MPa in the untreated controls (Wilson, 2001) ^[45]. It has been observed that commercial *A. nodosum* extract improves lettuce seedling performance when exposed to high temperatures. Additionally, it has been discovered that applying seaweed extracts to creeping bent grass increased its heat tolerance (Ervin *et al.*, 2004 ^[20]; Zhang and Ervin, 2008). The authors proposed that this effect was caused by the extract's "cytokinin-like" chemicals and increased K⁺ absorption.

Table 1: Effect of seaweed extract on vegetable

Crop	Target stresses	Seaweed Extracts	Improve traits	References
Spinach	Heat stress	<i>Ascophyllum nodosum</i>	Germination and seedling vigor	Anjos <i>et al.</i> (2020) ^[6]
Tomato	Water stress	<i>Ascophyllum nodosum</i> and <i>Laminaria digitata</i>	Increased the dry matter content, reduction in ABA accumulation.	Campobenedetto <i>et al.</i> (2021) ^[12]
Cucumber	Mineral stress	<i>Ulva lactuca</i> , <i>Jania rubens</i> , and <i>Pterocladia capillacea</i>	Growth, yield, NPK content	Hassan <i>et al.</i> (2021) ^[24]
Tomato	Salinity stress	<i>Padina gymnospora</i>	Early flowering, enhances fruit weight and quality	Hernández <i>et al.</i> (2022) ^[25]
Oktra	Salt stress	<i>Sargassum wightii</i>	Carbohydrates, proteins, fats, carotenoids, and proline and decreases the level of hydrogen peroxide and abscisic acid	Khan <i>et al.</i> (2022) ^[30]
Onion	Water stress	<i>Amphora ovalis</i>	Growth and yield	Almaroia and Eissa (2022)
Chicory	Drought stress	<i>Ecklonia maxima</i>	Yield, quality, ascorbic acid content	Sabatino <i>et al.</i> (2023) ^[39]

2. Humic substances

Humic substances comprise humic acids, fulvic acids and humins. Humic substances are components of soil organic matter, they are produced through microbial residue, plant, and animal decomposition as well as soil micro-organism metabolism (Rouphael and Colla, 2018). Humic acid helps in improving plant development by increasing bioavailability through root-soil environmental modification (Chen *et al.*, 2004). While fulvic acids increase soil water retention. These benefits can be attributed to improved water and nutrient absorption, increased resistance to environmental stress, and altered plant metabolism (Canellas *et al.*, 2015). The impact of

humic chemicals on plant physiology, however, is still up for discussion. This is because of the compounds' intricate molecular makeup as well as the variety and amount of plant reactions that are changed when they are applied. Humic and fulvic acids helps in mitigating both biotic and abiotic stresses. Bell pepper (*Capsicum annuum* L. cv. *Demre*) plants treated with humic acid and phosphorous had lower Na contents and higher ion contents in their roots and shoots of N, P, K, Ca, Fe, Mg, S, Mn, and Cu. These results were linked to a general protective effect against mild salinity stress (Cimrin *et al.*, 2010). Humic acid treatment of common beans (*Phaseolus vulgaris* L.) under high salinity (120 mM NaCl) resulted in

higher endogenous proline levels and decreased membrane leakage (Aydin *et al.*, 2012) ^[7], both of which are signs of improved saline environment adaption.

Table 2: Effect of Humic substances on vegetables

Crop	Targeted stresses	Humic substances	Improved traits	References
Onion	Water stress	Humic acid	leaf dry weight, vitamin C, calcium, total chlorophyll and bulb flavonoids	Forotaghe <i>et al.</i> (2022)
Broccoli	Salt stress	Humic acid	plant height, leaf area and head weight of broccoli per plant	Falahi <i>et al.</i> (2022) ^[3]
cucumber	Drought stress	Humic acid	Increases the nitrogen uptake by plants and stimulates and enhances the uptake of Ca, Mg, P, and K, increasing chlorophyll concentration, growth and yield.	Najafi <i>et al.</i> (2022) ^[34]
Broccoli	Water stress	Humic acid	Increased WUE, growth and yield	Ibrahim <i>et al.</i> (2024) ^[27]
Lettuce	Cadmium stress	Fulvic acid	increased the expression of Light-harvesting proteins, reaction center and electron transport-related proteins, enhanced sulphur metabolism and rebuilt redox homeostasis in cells	Chen <i>et al.</i> (2022) ^[14]
Chinese flowering cabbage	Calcium nitrate stress	Fulvic acid	Increased oxidation resistance, photosynthetic capacity and reduced Ca(NO ₃) ₂ accumulation	Wu <i>et al.</i> (2023) ^[46]
Tomato	Copper and cadmium stress	Fulvic acid	Increased the absorption and transport of essential nutrients, improve the appearance and flavour quality	Shi <i>et al.</i> (2023) ^[40]

3. Hydrolyzed Proteins and Nitrogen-Containing Compounds

Hydrolysed proteins can be derived from plant and animal sources using chemical, enzymatic, or thermal protein hydrolysis, or by combining these various hydrolysis methods. Hydrolysed proteins are a mixture of amino acids, peptides, polypeptides, and denatured proteins (Colla; Nardi). The enzymatic hydrolysis conditions have a considerable impact on the molecular weight, hydrophobicity, and polar groups of the proteins, which can determine the functional characteristics and bioactivities of protein hydrolysates. Enhancing the functional quality and bioavailability of food items requires attention to functional aspects of protein hydrolysates, such as solubility, emulsification, foaming, and other characteristics. Numerous research investigations on horticultural crops have shown that

applying protein hydrolyzates derived from both plants and animals can enhance crop development and resilience to abiotic challenges (Schiavon; Colla). Amino acid-rich extracts might contribute to an increased ability to withstand cold temperatures. After being exposed to cold temperatures and given an amino acid mixture called Terra-Sorb, which is produced by the enzymatic degradation of proteins, lettuce plants (*Lactuca sativa*) showed increased fresh weights and enhanced stomatal conductance (Botta, 2013) ^[9]. Tomato plants treated with Megafol under drought stress showed higher fresh weight and relative water content, suggesting a protective effect on water status and stress-responsive genes (Vasconcelos *et al.*, 2009 ^[17]; Petrozza *et al.*, 2014) ^[37]. Application of Megafol also enhanced the induction of several drought-responsive genes, including tomato orthologs of RAB18 and RD29B.

Table 3: Effect of protein and nitrogen-containing compounds on vegetables

Crop	Targeted stress	Protein hydrolysates	Improved traits	References
Tomato	Water stress	Pepton (animal protein)	Increased anti-oxidant protection, increases endogenous content of endole-3-acetic acid (auxin), trans-zeatin, and jasmonic acid	Casadesús <i>et al.</i> (2019) ^[13]
Capsicum	Drought stress	GHI_16_VHL, (plant protein hydrolysate)	Increased the relative growth rate and final fruit yield, and provides faster and more efficient plant recovery	Agliassa <i>et al.</i> (2021) ^[2]
French bean	Salt stress	Pumpkin seed protein hydrolysate	Reduction in malondialdehyde (MDA), increased growth and yield	Sitohy <i>et al.</i> (2022) ^[41]
Capsicum	Ozone-induced abiotic stress	Okra enzymatic extract	net photosynthetic rate, electron transport rate, effective quantum yield of PSII, delayed fluorescence, increased anti-oxidant enzymes in leaves	Ortiz <i>et al.</i> (2024)
Tomato	Drought stress	Vegetal protein from malvaceae and fabaceae	Enhanced plant resistance to water stress and increased the concentration of dipeptides and fatty acids and secondary metabolism	Leporino <i>et al.</i> (2024) ^[32]

4. Microorganisms

The principal members of this group include filamentous fungi, yeasts, and bacteria (Kour). They are kept apart from soil, vegetation, and other organic materials. They can increase crop productivity directly or indirectly and are applied to the soil or the seeds (Castiglione). Through nitrogen fixation and nutrient solubilisation, they improve nutrient uptake; they also induce the biosynthesis of plant hormones, such as auxins and cytokinins, to alter the hormonal status; they increase resistance to abiotic stresses and generate volatile organic compounds (VOCs), which may also directly affect plants. Plant responses to abiotic stressors can be improved by plant growth-promoting rhizobacteria (PGPR), which stimulate physical, chemical, and

biological activities (Ruzzi and Aroca, 2015; Turan *et al.*, 2017) ^[44]. Soils administered with Plant Growth-Promoting Rhizobacteria (PGPR) can reduce plant responses to abiotic stress. Rhizobium's ability to shield plants from abiotic stress has been thoroughly reviewed in several recent papers (Gopalakrishnan *et al.*, 2015) ^[22]. Tolerance to salt stress varies within these microorganisms and their tolerance can offer advantages to the host relationship under stress conditions. Two strains of Rhizobium leguminosarum, a salt-tolerant (GRA19) and salt-sensitive (GRL19) strain, were used to inoculate two legumes: pea (*Pisum sativum*) and fava bean (*Vicia faba*) (Del *et al.*, 1999) ^[18]. The plants inoculated with the salt-tolerant strain fared better under moderate salt stress.

Table 4: Effects of microorganisms on vegetables

Crop	Targeted stress	Micro organism	Improved traits	References
French bean	Water stress	Nomoren (20% arbuscular mycorrhizal fungi), EKOprop (arbuscular mycorrhizal fungi, rhizosphere symbiotic bacteria, and saprophytic fungi), and Twin-Antistress (<i>Bacillus subtilis</i> , as well as yeast) and <i>Ascochyllum nodosum</i>	increase the pod yield and the quality of green pods and seeds, helps in alleviation of negative effects of drought stress, and increased the total yield of green pods.	Petropoulos <i>et al</i> (2020) [36]
Tomato	Drought stress	<i>Azotobacter chroococcum</i> , <i>Azospirillum brasilense</i>	Restored the plant growth and enhanced organic matter and the total N,P,Ca, and Cu content of the experimental soil	Turan <i>et al</i> (2023) [44]
Tomato	Drought stress	arbuscular mycorrhizal fungi	improved photosynthetic efficiency, leaf water potential, and osmolytes, and reduced malondialdehyde (MDA) and hydrogen peroxide (H ₂ O ₂) accumulation	Soussani <i>et al</i> (2023) [42]
Tomato	Nitrogen and drought stress	<i>Kocuria rhizophila</i> and <i>Streptomyces violaceoruber</i>	improve the seed germination index, increased morpho-physiological parameters such as fresh and dry root weight, root diameter and lateral root numbers, chlorophyll content, net photosynthesis 1rate, transpiration and and improve NUE efficiency	Caldiero (2024) [11]

5. Inorganic compounds with biostimulant action

Beneficial elements are substances that can encourage plant growth without being necessary for all plants (Smits *et al.*, 2009; Jardin 2015). The elements aluminium, cobalt, sodium, selenium, and silicon are the five key beneficial elements. They support plant development, plant product quality, and resistance

to abiotic stress. These elements can be found in plants and soils as insoluble salts, such as the amorphous silica found in grasses (Savvas and Ntatsi; Azad). The advantageous outcomes that can be produced can be transient-for instance, after being exposed to specific abiotic stressors-or constitutive for example, strengthening of cell walls through silica deposits.

Table 5: Effect of inorganic compound with biostimulant action on vegetables

Crop	Targeted stress	Inorganic substance	Improved traits	References
Arugula	Drought stress	Selenium-based biostimulant	Increased water use efficiency, growth, yield and leaf area values	Mangarotti <i>et al</i> (2020)
Lettuce	Water stress	Silicon-based biostimulant	enhanced plant growth under water stress conditions, reduced lipid peroxidation and H ₂ O ₂ levels and protected photosynthesis performance.	Santiago <i>et al</i> (2021)
Tomato	Salt stress	sodium selenate	Improved morphometric and physicochemical characteristics of fruits such as weight, size, color, Brix degree, and lycopene content	Hajlaoui <i>et al</i> (2021) [23]
Tomato	Salt stress	Silicon-based biostimulant	Increased plant height, number of leaves and chlorophyll contents	Ghani <i>et al</i> (2021) [1]

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