

Biostimulants Application in Horticultural Crops under Abiotic Stress Conditions

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Abiotic stresses strongly affect plant growth, development, and quality of production; final crop yield can be really compromised if stress occurs in plants' most sensitive phenological phases. Additionally, the increase of crop stress tolerance through genetic improvements requires long breeding programmes and different cultivation environments for crop performance validation. Biostimulants have been proposed as agronomic tools to counteract abiotic stress. Indeed, these products containing bioactive molecules have a beneficial effect on plants and improve their capability to face adverse environmental conditions, acting on primary or secondary metabolism. Many companies are investing in new biostimulant products development and in the identification of the most effective bioactive molecules contained in different kinds of extracts, able to elicit specific plant responses against abiotic stresses. Most of these compounds are unknown and their characterization in term of composition is almost impossible; therefore, they could be classified on the basis of their role in plants. Biostimulants have been generally applied to high-value crops like fruits and vegetables; thus, in this review, we examine and summarise literature on their use on vegetable crops, focusing on their application to counteract the most common environmental stresses.

plant biostimulant

environmental stress

vegetables

1. Abiotic Stresses

Plants are continuously subjected to a multitude of stressful events, from seed germination through to the whole life cycle. These stresses are commonly divided into two categories—biotic and abiotic stresses—depending on the nature of the trigger factor. The first are caused by other living organisms, including insects, bacteria, fungi, and weeds that affect plant development and productivity. The second are generally linked with the climatic, edaphic, and physiographic components of the environment, when they are limiting factors of plant growth and survival. The most important abiotic stresses limiting agricultural productivity, almost all over the world, are drought, salinity, non-optimal temperatures, and low soil fertility. Among these, drought, and nutrient deficiencies are major problems, mostly in developing countries where the incomes of rural people depend on agriculture ^[1]. Actually, in “The State of Food and Agriculture 2007”, FAO reported that only 3.5% of the global land area is not affected by some environmental constraints. In 1982, Boyer estimated that yield losses caused by unfavourable environments were as much as 70% ^{[2][3]}. Farooq et al. ^[4] reported that drought induced a reduction of yield between 13% and 94% in several crops, depending on the intensity and duration of the stress. Afterwards, Cramer et al. ^[5] estimated the impacts of different abiotic stresses on crop production in terms of the percentage of global land area affected, considering the 2000 and 2007 FAO reports. They also referred to the increasing number of publications focused on this topic between 2001 and 2011. The exact impact of these changes on agricultural systems is extremely

difficult to predict and it depends on numerous parameters that are all not always included in predictive models. Even if some projections show that positive and negative outcomes on crop production could be balanced in the medium term, several studies agree that in the long term, the negative ones will prevail [6][7]. Based on future scenarios, adaptation and mitigation are essential to increase the resilience capacity of agricultural systems and to ensure crops yield and quality. Since environmental conditions cannot be controlled, several strategies on different levels are required, such as agronomical techniques or breeding of more tolerant cultivars [8].

In 2010, at the society's annual conference, Vegetable Breeding and Stress Physiology working groups of the American Society for Horticultural Sciences focused particularly on the "Improvement of Horticultural Crops for Abiotic Stress Tolerance" considering the effects of climate change [9]. Up to now, most studies on climate change impacts focus on major crops, and only few papers pay attention to fruit and vegetable in terms of production, quality, and supply chain [10][11]. An important aspect to take into consideration is the effect of the combination of different stressful factors. Most of the time, crops are subjected to several abiotic stresses that occur simultaneously in the field. In these situations, studying the stresses separately is not enough because plant response is unique and cannot be predicted by the reply obtained when each factor is applied individually [12][13][14]. Moreover, biotic and abiotic components typically interact in an ecosystem. For instance, environmental conditions affect plant-pest interaction in different ways, by decreasing plant tolerance or increasing the risk of pathogen infection [15][16].

Focusing on horticultural species, the tolerance to abiotic stresses is an important trait because their cash value is usually higher than field crops, they require more resources for farming and because they provide a source of many nutrients, fibre, minerals, and carbohydrates, which are essential in a healthy diet [17]. Food and Agriculture Organization (FAO) reports that about 90% of essential vitamin C and 60% of vitamin A for human comes from vegetables. Indeed, low fruit and vegetable intake is a major contributing risk factor to several widespread and debilitating nutritional diseases. According to the Global Burden of Disease Study, 3.4 million deaths can be attributed to low consumption of fruit and 1.8 million to low vegetables diets worldwide [18]. Therefore, growing high-quality vegetables becomes one of the most important goals of current agriculture, in order to meet the needs of the population and the increasing demand for fruit and vegetables. Abiotic stresses do not only affect the yield but also the quality of these products, triggering morphological, physiological and biochemical changes that can alter the visual appearance and/or the nutraceutical value in a way that the product could become unmarketable [19]. Bisbis et al. [11] investigated the double effects of elevated temperature and increased CO₂ on the physiology of different vegetables. They observed several responses according to plant species and severity of the stress, taking into consideration the possible adaptation strategies that could be implemented in order to mitigate the effects of climate change. Nonetheless, these mechanisms are still under-researched and should be studied in depth, because not only different species but different cultivars also could respond differently to the same environmental stress. For example, cultivars with low levels of antioxidants are particularly vulnerable to oxidative stress compared to those with high antioxidant activity [20][21][22][23]. This aspect has a particular importance as selection criterion in the choice of appropriate cultivars for a specific situation. Oxidative stress is a common phenomenon caused by several adverse conditions; it generally occurs when the balance between the production of reactive oxygen species (ROS) and the quenching activity is upset by a stressful event [24]. Low levels of ROS are normally

produced by different reactions during physiological metabolisms like photosynthesis or respiration, and they play an important signaling role in plant growth and development. Their amount dramatically increases under abiotic stress conditions and, if not controlled could result in cellular damage and death. Besides their toxicity to proteins, lipids or nucleic acids, the increased production of ROS under stressful conditions plays a key role in the complex signaling network of plants stress responses. Their concentration is maintained at non-toxic levels by the activity of the antioxidant system: a wide range of enzymatic or non-enzymatic antioxidant molecules are accumulated in plant tissues to quench ROS induced by stress [\[25\]\[26\]\[27\]\[28\]](#). Moreover, the maintenance of this equilibrium is also dependent on numerous factors, such as the timing of stress application, its intensity and duration. Indeed, moderate or controlled stress conditions could have a positive effect on quality traits of several crops [\[29\]](#). For example, water deprivation might be a useful crop management strategy to improve the quality of lettuce and fleshy fruits in terms of nutritive and health-promoting value and taste, by stimulating the secondary metabolism and concentration of different phytochemicals such as α -tocopherol, β -carotene, flavonoid and so on [\[30\]\[31\]](#). Besides the production of ROS scavenging compounds, plants also increase the biosynthesis and accumulation of compatible solutes with an osmoprotective role, like sugars and proline.

Plants generally reply to non-optimal environmental conditions both with short- and long-term adaptation strategies, by the activation and regulation of the expression of specific stress associated genes [\[32\]\[33\]](#).

Since plants are sessile organisms and they have to cope with adverse external conditions; all these mechanisms are essential for their survival. These strategies are effective if they are activated in time, in order to set a defense response and anticipate the environmental changes that might affect plant growth irreversibly. The trade-off between growth and acclimation metabolisms results in a sort of fitness cost for plants, since energy and nutrients normally destined to growth and production are intended for stress responsive mechanisms [\[34\]](#).

Agronomic management conducted in order to enhance plant tolerance towards abiotic stresses evolved over the centuries due to the technologic progress, climate change, scientific knowledge, and farmers' experiences. The choice of the correct cultivar, the best growing period, the sowing density, and the amount of water or fertilizers are some of the most common strategies applied to mitigate the negative effects of abiotic stresses [\[8\]](#). Protected cultivation is a cropping technique adopted to preserve plants from unfavourable outdoor conditions. It is mainly suited to vegetables and floriculture production in a non-optimal environment, through the control of temperatures, radiation or atmospheric composition. Another agronomical strategy, especially applied in vegetable crops, is soilless cultivation. This approach allows controlling of water and nutrients, avoiding the use of soil for cultivation and all the problems related to it, like poor quality or contamination.

Grafting is an additional tool adopted to counteract environmental stresses and increase tolerance in vegetable crops. This technique is applied especially to high-yielding fruits and vegetables such as cucurbits and solanaceous to enhance tolerance against saline soil, nutrient or water deficiency, heavy metals or pollutants toxicity [\[35\]\[36\]\[37\]](#).

Agronomical strategies are essential in mitigating the negative effect of several abiotic stresses, but sometimes their application is not enough. Moreover, current experiments aim to transfer one or more genes involved in signaling or regulatory pathways, or genes encoding to molecules, such as osmolytes and antioxidants, conferring tolerance to a specific abiotic stress [38]. Several functional and regulatory genes involved in abiotic stress tolerance have been identified and studied. Results of these studies can be exploited for genetic improvement aiming to introduce tolerance traits in cultivated crops. Since different physiological traits related to stress tolerance are under multigenic control, the manipulation of a single gene generally is not enough. Hence, scientists have paid more attention to regulatory genes, including transcription factors, due to their ability to regulate a vast array of downstream stress-responsive genes at a time [39][40][41].

However, the huge existing genetic variability among vegetable species, the lack of knowledge about minor cultivars genome, the complex responses triggered by abiotic stress conditions and the limited strategies currently available make genetic improvement really difficult and often inefficient. Moreover, besides the wide diversity of germplasms available, plant tolerance to stress depends both on stress features such as duration, severity, and frequency, as well as the affected tissues and development stages of crops [24][42][43][44].

Additionally, the increase of crop tolerance through genetic improvements requires many years of work and different cultivation environments that cannot be always taken into consideration. As a result, several new cultivars that can be used by the growers are released each year.

Another technique widely used for developing stress tolerance in plants is in vitro selection. This culture-based tool allows better understanding of several plants' physiological and biochemical responses to adverse environmental conditions. It has been applied specially to obtain salt/ and drought/tolerant lines in a wide range of plant species, including vegetables [45]. In vitro selection is based on the induction of a genetic variation among cells, tissues or organs, their exposure to a stressor, and the subsequent regeneration of the whole organism starting from the surviving cells [46]. Even if in vitro selection is a less expensive and time-saving approach compared with classic molecular engineering, some limitations, mostly concerning the stability of the selected traits and epigenetic adaptation, still exist.

In addition to these strategies, it has been observed that stress tolerance can also be induced by biostimulants or specific bioactive compounds, if they are applied on vegetable crops when they really need to be protected [47][48][49]. Biostimulant application on horticultural crops under environmental stress conditions will be discussed in detail below.

2. Biostimulants

Biostimulant products have been considered innovative agronomic tools as demonstrated by the increase of scientific publications and by the constant expansion of their market [50]. France, Italy, and Spain are the leading EU countries in the production of biostimulants [51]. According to a new report by Grand View Research, Inc., the biostimulant market size is expected to reach USD 4.14 billion by 2025 [52]. The complex nature of the composition

of these products and the wide range of molecules contained makes it complicated to understand and define which compounds are the most active. The isolation and study of a single component is almost impossible and the efficacy of a biostimulant is not due to a single compound but is the consequence of the synergistic action of different bioactive molecules. Moreover, the application rules and time are not always clear. For all these reasons, the European Commission developed a proposal for a new regulatory framework and a draft for a new fertilizer regulation was prepared in 2016. The amendments to the proposal of the European Commission were adopted by the European Parliament in October 2017, while the legislative resolution on the proposal was approved on 27 March 2019 [53][54][55].

Plant biostimulants are defined as products obtained from different organic or inorganic substances and/or microorganisms, that are able to improve plant growth, productivity and alleviate the negative effects of abiotic stresses [56][57]. Mineral elements, vitamins, amino acids, and poly- and oligosaccharides, trace of natural plant hormones are the most known components. However, it is important to underline that the biostimulant activity must not depend on the product's nutrients or natural plant hormones content. The mechanisms activated by biostimulants are often difficult to identify and are still under investigation [58]. High-throughput phenotyping and omic technologies seem to be useful approaches to understand biostimulants activity and hypothesize a mode of action [59][60][61]. They can act directly on plant physiology and metabolism by improving soil conditions [62][63]. They are able to modify some molecular processes that allow to improve water and nutrient use efficiency of crops, stimulate plant development, and counteract abiotic stresses [47] by enhancing primary and secondary metabolism [55][61][63].

One of the key points of the discussion is about the application of these products in stressful conditions and their role as nutrients, not with a curative function. In particular, if a product has a direct effect against biotic stresses, it should not be included in the biostimulant category but should be registered as plant protection products.

2.1 Biostimulants and Crop Tolerance to Abiotic Stresses

Table 1 is a summary of biostimulant products or bioactive molecules from different origins that have been evaluated for amelioration of abiotic stresses in several vegetables species. The biostimulants effectiveness to counteract the stressful condition depends on several factors, such as timing of application and their mode of action. The application of biostimulants can be carried out with different timings: before the stress affects the cultivation, during the stress, or even after. They could be applied on seeds, when plants are in early stages of growth, or when crops are fully developed, depending on the desired results [64]. As general consideration, biostimulants that contain anti-stress compounds, such as proline or glutamic acid, can be applied when the stress occurs or during stress conditions. On the contrary, those that are involved in the activation of bioactive compounds biosynthesis must be applied before the stress occurs. Proper timing of application during crop development differs from species to species and it also depends on the most critical phases for crop productivity. Thus, the identification of the right time of biostimulant application is as important as the determination of the exact dose, in order to avoid waste of product, high production costs, and unexpected results. Biostimulants can be applied as foliar spray or to the roots, at sowing for protecting the seedling in the early development stages, in a floating

system nutrient solution or during blooming or fruit setting. There is no general recipe that works for a crop species and in each stress situation.

The protective role of biostimulants on plants has been increasingly studied. These products are able to counteract environmental stress such as water deficit, soil salinization, and exposure to sub-optimal growth temperatures in several ways [47][56][65][66]. They improve plant performance, enhance plant growth and productivity, interact with several processes involved in plant responses to stress, and increase the accumulation of antioxidant compounds that allow decrease in plant stress sensitivity.

Table 1. Examples of biostimulant products or substances with a biostimulant effect on horticultural crops to counteract abiotic stress conditions.

ABIOTIC STRESS	SEVERITY AND TIME OF EXPOUSURE	BIOSTIMULANT PRODUCT OR SUBSTANCES WITH A BIOSTIMULANT EFFECT	DOSE	APPLICATION METHODS AND NUMBER OF TREATMENTS	CROP	BENEFICIAL EFFECTS	REFERENCE
Chilling or cold stress	6 °C for 6 days	Asahi SL (Sodium para-nitrophenolate, sodium ortho-nitrophenolate, sodium 5-nitroguaiacolate) / Goëmar Goteo (Composition (w/v): organic substances 1.3–2.4%, phosphorus (P ₂ O ₅) 24.8%, potassium (K ₂ O) 4.75%)	0.1%	Foliar spray (3)	<i>Coriandrum sativum</i> L.	Chlorophyll a and carotenoids ↑Fv/Fm ↑E ↑gs ↓Ci	[65]
	10, 12 °C for 7 days / 15 °C for 7, 10 days	<i>Flavobacterium glaciei</i> , <i>Pseudomonas frederiksbergensis</i> , <i>Pseudomonas vancouverensis</i>	-	Seed inoculation	<i>Solanum lycopersicum</i>	↑shoot height ↑root length ↑biomass accumulation ↓electrolyte leakage ↓lipid peroxidation ↑proline accumulation	[67][68]

					↑SOD, CAT, APX, POD, GR activity	
−6 °C for 5 nights	Pepton 85/16 (enzymatic hydrolysates obtained from animal haemoglobin. L-α amino acids (84.83%) and free amino acids (16.52%), organic-nitrogen content (12%), mineral-nitrogen content (1.4%), potassium content (4.45%), iron content (4061 ppm), very low heavy-metal content)	2 L ha ^{−1} , 4 L ha ^{−1}	Injection into the soil (5x)	<i>Fragaria × ananassa</i>	↑new roots ↑flowering ↑fruit weight	[69]
−3 °C for 4 h	Pepton 85/16	0.4, 0.8, 1.6 g L ^{−1}	Soil application (1x)	<i>Lactuca sativa</i> L.	↑fresh and dry weight ↑SLA ↑RGR	[70]
4 °C for 8 days or nights /6 °C for 8 days only to the roots	Terra-Sorb® Foliar (Free amino acids (ASP, SER, GLU, GLY, HIS, ARG, THR, ALA, PRO, CIS, TYR, VAL, MET, LYS, ILE, LEU, PHE, TRP) 9,3% (w/w), Total amino acids 12% (w/w), Total nitrogen (N) 2,1% (w/w), Organic Nitrogen (N) 2,1% (w/w), Boron (B) 0,02% (w/w), Manganese (Mn) 0,05% (w/w), Zinc (Zn) 0,07% (w/w), Organic matter 14,8% (w/w))	3 mL L ^{−1}	Foliar spray (3x)	<i>Lactuca sativa</i> L. var. <i>capitata</i>	↑roots fresh weight ↑green cover %	[71]
3 °C for 48 h	5-aminolevulinic acid	0, 1, 10, 25, 50 ppm (15	Seed soaking/ foliar spray/soil	<i>Capsicum annuum</i>	↓visual injuring ↑chlorophyll	[72]

			mL for seed soaking and 25 mL for soil drench)	drench (1x)		↑RWC ↑gs ↓membrane permeability ↑shoot and root mass ↑SOD activity	
Drought stress	Occlusion of xylem vessels	<i>Azospirillum brasilense</i> (BNM65)	-	Seed inoculation	<i>Solanum lycopersicum</i>	↑height plants ↑dry weight ↑xylem vessel area	[73]
	No irrigation for 5 days	Megafol® (Composition (w/v): total nitrogen (N) 3.0% (36.6 g L ⁻¹); organic nitrogen (N) 1.0% (12.2 g L ⁻¹); ureic nitrogen (N) 2.0% (24.4 g L ⁻¹); potassium oxide (K ₂ O) soluble in water 8.0% (97.6 g); organic carbon (C) of biological origin 9.0% (109.8 g L ⁻¹))	2 mL L ⁻¹	Foliar spray (1x)	<i>Solanum lycopersicum</i>	↑leaf area ↑RLWC	[74]
	50% ET	<i>Ascophyllum nodosum</i>	0.50%	Foliar spray and drench	<i>Spinacia oleracea</i>	↑RLWC ↑leaf area ↑fresh and dry weight ↑SLA ↑gas exchange	[75]
	No irrigation until symptoms of wilting appear	<i>Pseudomonas</i> spp. (<i>P. putida</i> <i>P. fluorescens</i>)	-	Seed inoculation	<i>Pisum sativum</i>	↑grain yield ↑root growth ↑shoot length ↑number of pods per plant ↑chlorophyll	[76]

No irrigation for 12 days	<i>Achromobacter piechaudii</i> (ARV8)	-	Seedling inoculation	<i>Solanum lycopersicum</i>	↑ fresh and dry weight of seedling ↑ plant growth ↓ ethylene	[77]
No irrigation for 12 days	<i>Achromobacter piechaudii</i> (ARV8)	-	Seedling inoculation	<i>Capsicum annuum</i>	↑ fresh and dry weight of seedling ↑ plant growth	[77]
No irrigation for 7 days	<i>Ascophyllum nodosum</i>	0.33%	Foliar spray (2x)	<i>Solanum lycopersicum</i>	↑ RWC ↑ plant growth ↑ foliar density ↑ chlorophyll ↓ lipid peroxidation ↑ proline ↑ soluble sugars	[78]
No irrigation for 2 days	<i>Ascophyllum nodosum</i> + amino acids	-	Soil application (1x)/ foliar spray (3x)	<i>Brassica oleracea</i> var. <i>italica</i>	↑ Pn ↑ gs ↑ chlorophyll	[79]
40, 70% field capacity	Gibbrellic acid and titanium dioxide	250, 500 ppm (GA3) 0.01, 0.03% (titanium nanoparticles)	Stems and foliar spray (2x)	<i>Ocimum basilicum</i>	↑ CAT activity ↓ lipid peroxidation ↑ LRWC	[80]
No irrigation	VIVA®	-	2x	<i>Solanum lycopersicum</i>	↑ plant biomass ↑ roots biomass	[81]
60, 40% field capacity	<i>Pseudomonades</i> , <i>Bacillus lentus</i> , <i>Azospirillum brasiliense</i>	-	Seed inoculation	<i>Ocimum basilicum</i>	↑ CAT, GPX activity ↑ chlorophyll	[82]

	60, 40% ET	Moringa leaf extract	3%	Foliar spray (2x)	<i>Cucurbita pepo</i>	↑growth ↑HI ↑WUE ↑Fv/Fm ↑PI ↑soluble sugars ↑free proline ↓electrolyte leakage ↑membrane stability	[83]
Heat stress	35 °C	Nano-TiO ₂	0.05, 0.1, 0.2 g L ⁻¹	Foliar spray (1x)	<i>Solanum lycopersicum</i>	↑gs ↑E ↑ Pn	[84]
	40/30 °C for 8 days	Brassinosteroids	0.01, 0.1, and 1.0 mg L ⁻¹	Foliar spray (1x)	<i>Solanum lycopersicum</i>	↑antioxidant enzyme activities ↓H ₂ O ₂ ↓MDA ↑shoot weight	[85]
	35.2 °C (Tmax)	Brassinosteroids	25, 50, 100 ppm	Foliar spray (2x)	<i>Phaseolus vulgaris</i>	↑plant length ↑number of leaves, branches and shoots per plant ↑fresh and dry weight ↑pod weight ↑N, P, K in bean pods	[86]
	45 °C for 90 min	Nitric oxide	150 μM	Immersion of leaf disks	<i>Phaseolus radiatus</i>	↑Fm ↓electrolyte leakage	[87]
	35/25 40/30 45/35 °C	Ascorbic acid	50 μM	In a nutrient solution	<i>Phaseolus radiatus</i>	↑% germination ↑seedling growth ↓electrolyte leakage ↑TTC reduction ability ↑RLWC ↓MDA	[88]

							↓H ₂ O ₂ ↑antioxidant activity ↑ascorbic acid ↑GSH ↑proline
	35/25 40/30 45/35 °C	Proline	5, 10, 15 μM	In a nutrient solution	<i>Cicer arietinum</i>		↑% germination ↑shoot and root length ↓electrolyte leakage ↑chlorophyll ↑RLWC ↓lipid peroxidation ↓H ₂ O ₂ ↑GSH ↑proline
						[89]	
	35/25 40/30 45/35 °C for 10 days	Absciscic acid	2.5 μM	In a nutrient solution	<i>Cicer arietinum</i>		↑shoot length ↑osmolytes ↑chlorophyll ↑cellular oxidizing ability
	42 °C for 48 h	Glutathione	0.5 mM	-	<i>Vigna radiata L.</i>		↑RLWC ↑chlorophyll ↑proline ↓MDA ↓H ₂ O ₂ ↓O ₂ ⁻ ↓LOX activity ↑ascorbate ↓GSSG
Heat and salt stress	35 °C and 75 mM NaCl for 15 days	Melatonin	100 μM	Foliar spray (5x)	<i>Solanum lycopersicum</i>		↑biomass ↑Pn ↑gs ↑E ↑chlorophyll a ↑carotenoids ↑Fv/Fm ↑efficiency of

						PSII ↑ETR ↑antioxidant capacity ↓H ₂ O ₂ ↓lipid peroxidation ↓protein oxidation
Iron deficiency	Actiwave® (<i>Ascophyllum nodosum</i>)(Composition (w/v): total nitrogen (N) 3.0% (38.7 g L ⁻¹); organic nitrogen (N) 1.0% (12.9 g L ⁻¹); ureic nitrogen (N) 2.0% (25.8 g L ⁻¹); potassium oxide (K ₂ O) soluble in water 7.0% (90.3 g L ⁻¹); organic carbon (C) of biological origin 12% (154.8 g L ⁻¹); iron (Fe) soluble in water 0.5% (6.45 g L ⁻¹); iron (Fe) chelated by ethylenediaminedi (2- hydroxy-5- sulfophenylacetic) acid (EDDHSA) 0.5% (6.45 g L ⁻¹); zinc (Zn) soluble in water 0.08% (1.03 g L ⁻¹); zinc (Zn) chelated by Ethylenediaminetetraacetic acid (EDTA) 0.08% (1.03 g L ⁻¹))	10 mL in 20 mL tap water	In a nutrient solution	<i>Fragaria ananassa</i>		↑vegetative growth ↑chlorophyll ↑stomatal density [92] ↑photosynthetic rate ↑ fruit production ↑berry weight
-	Amino acids	0.1, 0.2 mL L ⁻¹ / 0.2, 0.7 mL L ⁻¹	Root application/foliar spray (4x)	<i>Solanum lycopersicum</i>		↑plant growth [93] ↑root and leaf ferrum chelate reductase activity

						↑chlorophyll Fe ↑Fe ₂ :Fe ratio
Reduced NPK	NPK reduced of 40%	VIVA® (Composition (w/v): total nitrogen (N) 3.0% (37.2 g L ⁻¹); organic nitrogen (N) 1.0% (12.4 g L ⁻¹); ureic nitrogen (N) 2.0% (24.8 g L ⁻¹); potassium oxide (K ₂ O) soluble in water 8.0% (99.2 g L ⁻¹); organic carbon (C) of biological origin 8.0% (99.2 g L ⁻¹); iron (Fe) soluble in water 0.02% (0.25 g L ⁻¹); iron (Fe) chelated by EDDHSA 0.02% (0.25 g L ⁻¹)	10.5 mL /plant	Foliar spray	<i>Solanum lycopersicum</i>	↑yield ↑ascorbic acid ↑lycopene ↑chlorophyll ↑carotenoids [94]
	NPK deprivation	Kelpak (<i>Ecklonia maxima</i> , containing polyamine, cytokinins and auxins, putrescine, spermine)	0.40%	In a nutrient solution (twice per week for 8 weeks)	<i>Abelmoschus esculentus</i>	↑number of leaves ↑number of roots ↑stem thickness ↑shoot weight ↑root weight ↑leaf area [95]
	NPK reduced of 50%	Bio-Cozyme (concentrated micro-biological biostimulant and soil inoculants. Total Nitrogen (N) 0.20%, Soluble Potash (KO) 5.00%, Magnesium (Mg) 1.40%, Boron (B) 0.20%, Copper (Cu) 0.50%, Iron (Fe) 3.00%, Manganese (Mn)1.00%, Molybdenum (Mo) 0.0.25%, Zinc (Zn) 2.00%,	2 kg ha ⁻¹	Foliar application (4x)	<i>Allium sativum</i>	↑bulb yield ↑plant height ↑NPK in leaves [96]

		Humic Acid, humates & derivatives 8.00%, Vitamins, E, C, B Complex, organic acids, natural sugars carbohydrates, amino acids 1.40%)				
Salt stress	30, 50, 80 mol m ⁻³ NaCl for 30 days / 40, 80, 120 mol m ⁻³ NaCl	<i>Azospirillum brasilense</i>	-	Seed inoculation	<i>Lactuca sativa</i>	↑germination % ↑total fresh and dry weight ↑biomass partition ↑plantlets number ↑plantlets dry weight ↑total leaf fresh weight ↑leaf area ↑leaves number ↑chlorophyll ↑root dry weigh ↑ascorbic acid ↑plant survival after transplant
	40, 80, 120 mM NaCl	<i>Azospirillum brasilense/Pantoea dispersa</i>	-	Inoculation	<i>Capsicum annuum</i>	↑plant dry weight ↑K ⁺ :Na ⁺ ratio ↑gs ↑relative growth rate ↑net assimilation rate ↓ Cl ⁻ accumulation ↑NO ₃ ⁻ concentration ↑CO ₂ assimilation

714 mg·L ⁻¹ NaCl	<i>Azospirillum brasilense</i> (ATCC 29,729)	-	Soil inoculation	<i>Cicer arietinum</i>	↑ nodule formation ↑ shoot dry weight	[100]
100 mmol L ⁻¹ NaCl	<i>Rhizobium leguminosarum</i> (GRA19–GRL19)	-	Seedling inoculation	<i>Vicia faba</i> / <i>Pisum sativum</i>	↑ plant growth	[101]
50, 100 mM NaCl	<i>Bacillus species, Bacillus pumilis, Trichoderma harzannum, Paenibacillus azotoformans and polymyxa</i>	-	Seed treatment/ watering	<i>Cucurbita pepo</i>	↑ fresh weight ↑ potassium uptake ↓ sodium uptake ↑ K ⁺ :Na ⁺ ratio	[102]
30, 60, 120 mM (NaCl, Na ₂ SO ₄ , CaCl ₂ , CaSO ₄ , KCl, K ₂ SO ₄ , MgCl ₂ , MgSO ₄) for 60 days	Humic acid	0.05, 0.1%	Soil application	<i>Phaseolus vulgaris</i>	↑ plant nitrate, nitrogen and phosphorus ↓ soil electricity conductivity ↓ proline ↓ electrolyte leakage ↑ plant root and shoot dry weight	[103]
-	Acadian (<i>Ascophyllum nodosum</i>)	-	Soil application	<i>Fragaria ananassa</i>	↑ yield ↑ growth ↑ root length ↑ surface area, volume and number of tips ↑ numbers of crowns	[104]
80 mM NaCl	Super Fifty® (<i>Ascophyllum nodosum</i>)	0.4, 1, 2.5, 10 mL L ⁻¹	In the nutrient solution	<i>Lactuca sativa</i>	↑ root, stem, total plant weight	[105]

25 mM NaCl	Protein hydrolysates	2.5 mL L ⁻¹	Foliar spray/soil application	<i>Lactuca sativa</i>	↑fresh yield ↑dry biomass ↑root dry weight ↑plant nitrogen metabolism ↑Fv/Fm ↓oxidative stress ↑osmolytes ↑glucosynolates	[106]
0.8, 1.3, and 1.8 dS/m NaCl	Retrosal® (organic mix with high concentration of carboxylic acids, containing calcium oxide (CaO) 8.0% (w/w) soluble in water and 1.4% complexed by ammonium ligninsulfonate, Zinc (Zn) 0.2% (w/w) soluble in water and 0.2% (w/w) chelated by EDTA.)	0.1 or 0.2 mL/plant	Soil application (4x)	<i>Lactuca sativa</i>	↑fresh weight ↑chlorophyll Pn ↑ gas exchange ↓proline ↓ABA	[107]
43, 207 mM NaCl for 7 weeks	<i>Achromobacter piechaudii</i>	-	Seedling inoculation	<i>Solanum lycopersicum</i>	↑fresh and dry weights of tomato seedlings ↓ethylene ↑uptake phosphorous and potassium ↑WUE	[108]
200 mM NaCl	Nano-TiO ₂	5, 10, 20 and 40 mg L ⁻¹	Foliar spray	<i>Solanum lycopersicum</i>	activities of carbonic anhydrase, nitrate reductase, SOD and POX ↑proline	[97]

						↑glycinebetaine ↑growth ↑yield	
	28, 56 mmol kg ⁻¹	<i>Ascophyllum nodosum</i>	1, 2 g kg ⁻¹	Soil application	<i>Cucumis sativus</i>	↑fruit yield ↑Pn	[109]
	7.15, 7.2 dSm ⁻¹	Licorice root extract	0.50%	Seed soaking /foliar spray	<i>Phaseolus vulgaris</i>	↑plant growth ↑yield ↑RWC ↑chlorophylls ↑free proline ↑total soluble carbohydrates ↑total soluble sugars ↑nutrients ↑selenium ↑K ⁺ :Na ⁺ ratio ↑membrane stability index ↑activities of all enzymatic antioxidants ↓electrolyte leakage ↓MDA ↓Na ⁺ ↓H ₂ O ₂ ↓O ₂ ⁻	[110]
	100 mM NaCl	Propolis and maize grain extract	1, 2%	Soaking seed	<i>Phaseolus vulgaris</i>	↑% germination ↑seedling growth ↑cell membrane stability index ↑RWC ↑free proline ↑total free amino acids ↑total soluble sugars ↑indole-3- acetic acid ↑gibberellic acid ↑activity of the antioxidant	[111]

					system ↓lipid peroxidation ↓electrolyte leakage ↓ABA
6.23–6.28 dS m ⁻¹	Salycilic acid and <i>Moringa oleifera</i>	0.30%	Seed soaking /foliar spray	<i>Phaseolus vulgaris</i>	↑shoot length ↑number and area of leaves ↑ plant dry weight ↑ ↑RWC ↑chlorophyll ↑carotenoid ↑total soluble sugars ↑free proline ↑ascorbic acid ↑N, P, K and Ca, ↑ratios of K/Na and Ca/Na ↑green pod and dry seed yields
100 mM NaCl	<i>Moringa oleifera</i>	crude extract	Soaking seed	<i>Phaseolus vulgaris</i>	↑shoot and root lengths ↑plant dry mass ↑total soluble sugars ↑proline ↑K ⁺ , Na ⁺ and Cl ⁻ ↑ascorbic acid ↑total glutathione ↓MDA ↓ H ₂ O ₂ ↓O ₂ ⁻ ↑SOD, APX, GR
50, 150 mM NaCl	<i>Sargassum muticum</i> and <i>Jania rubens</i>	1%	Foliar spray (2x)	<i>Cicer arietinum</i>	↑plant growth ↑chlorophyll ↑carotenoid ↑soluble sugars ↑phenols ↓Na ⁺ ↑K ⁺ ↓H ₂ O ₂ ↑CAT,

						SOD, POD, APX activity ↓MDA
	3, 6 g L ⁻¹	<i>Dunaliella salina</i> exopolysaccharides	0.1 g L ⁻¹	Foliar spray (2x)	<i>Solanum lycopersicum</i>	↑chlorophyll ↑protein ↓proline [116]
	8.81 dS m ⁻¹	Bee-honey based biostimulant	25–50 g L ⁻¹	Foliar spray	<i>Allium cepa</i>	↑biomass ↑bulb yield ↑WUE ↑photosynthetic pigments ↑osmoprotectants ↑membrane stability index ↑RWC ↑enzymatic and non-enzymatic antioxidants [117]
	8 mM NaCl	phosphorus / humic acid	50, 100, 150 mg kg ⁻¹ (P)/750, 1500 mg kg ⁻¹ (humic acid)	Soil application	<i>Capsicum annuum</i>	↑fresh and dry weight of shoot and root ↓membrane damage ↑nutrient uptake [118]
UV-stress	300–340nm illumination for 15 min	Nano-anatase	0.25%	Soaking seed and foliar spray	<i>Spinacia oleracea</i>	↓O ₂ ↓H ₂ O ₂ ↓MDA ↑SOD, CAT, APX, GPX activity [119]

Fv/Fm maximum quantum efficiency of Photosystem II; Pn net photosynthetic rate; E transpiration rate; gs stomatal conductance; Ci sub stomatal CO₂ concentration; SLA specific leaf area; RGR relative growth rate; RLWC relative leaf water content; RWC relative water content; WUE water use efficiency; PI performance index; MDA malondialdehyde; TTC 2,3,5-triphenyltetrazolium chloride; GSH reduced glutathione; GSSG oxidized glutathione;

LOX lipoxygenase; CAT catalase; SOD superoxide dismutase; APX ascorbate peroxidase; POX peroxidase; GR glutathione reductase; HI harvest index; ABA abscisic acid; ETR electron transport rate. The symbol ↑ means an increase or ↓ a decrease of the parameter measured. The symbol x represents how many times the treatment was applied.

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