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Role of Biostimulants in Agriculture

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Abstract

In agriculture, agrochemicals play a vital role in enhancing yield and productivity of crops under optimal and suboptimal conditions. In recent times, different innovations have been projected to reduce the use of fertilizers and pesticides and enhance sustainable agricultural production. An environment-friendly technique has been proposed which include the use of natural plant biostimulants. Biostimulants boost the growth and development from germination to maturity throughout the life cycle of a crop, increase the plant metabolism efficiency for higher yield and crop quality, increase tolerance against abiotic stresses, facilitates nutrient use efficiency and translocation, improves the quality of produce (colour, total sugar content, fruit seeding), efficient use of water, and improves the physiochemical properties of the soil. Plant bio stimulants include the substances which are applied to soil, plant, or seed in precise formulations to alter the physiological processes which, in turn, enhance the growth and development, fruit set, nutrient uptake, and stress responses in plants. Various extracts from algae or plant, protein hydrolysates, humic acid, fulvic acid, and other mixtures are used as biostimulants in plants. These substances can be directly added to soil in the form of soil preparations or as liquid foliar application products. Plant biostimulants were earlier used only in organic production, but now they are also used in conventional and integrated crop production systems.

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10.1 Introduction

Plant biostimulants, also known commonly as agricultural biostimulants, are a diverse class of compounds which can be applied to a plant which not only positively affect growth and nutrient uptake but also provide tolerance against various stresses. Biostimulants are applied to the root zone of plant to enhance nutrient uptake and protect the plant against drought stress, salinity, and temperature stress (Jardin 2015). Though biostimulants are not nutrients, they facilitate the uptake of nutrients or beneficially contribute to growth and development or stress resistance of plant (Brown and Saa 2015). Now it is a well-established fact that plants are not standalone entities but are hosts and help many types of microorganisms, hosting several microbiota and their inter-connection in plant tissues and help in adaption in various stress environments (Vandenkoornhuyse et al. 2015). The agricultural sector is facing huge challenges for increasing the yield to meet the demands of ever-increasing population with less environmental influence on human health. Fertilizers and pesticides play a vital role in augmenting yield and productivity in agriculture throughout the growing seasons under different conditions. So recently, various innovations have been proposed for sustainable agriculture production with lesser use of synthetic agrochemicals. Environment-friendly technique includes natural biostimulants which will increase yield, flowering, fruit development, productivity, and nutrient uptake and enhance tolerance against various abiotic and biotic stresses (Colla and Rouphael 2015). Plant biostimulants are heterogeneous materials comprising different categories of materials which act as biostimulants such as humic substances and fulvic acid, microbial inoculants, complex organic substances, inorganic salts, beneficial chemical elements, seaweed extract, anti-transpirants, protein hydrolysates, and amino acids (du Jardin 2012). According to new regulation (EU) 2019/1009, a plant biostimulant shall be an EU fertilizing material which helps to stimulate plant nutrition uptake and also one or more other characteristics of the plant rhizosphere such as nutrient use efficiency, abiotic stress tolerance, and nutrient uptake in the soil (EU 2019). Plant biostimulants help in disease resistance by focusing the effects on growth and stress tolerance. Biostimulants were categorized as “substances, comprising microorganisms which are applied to plant, seed, soil, or other growing media that may enhance the plant’s ability to assimilate applied nutrients, or provide benefits to plant development in North America. Biostimulants are not plant nutrients so may not make any nutrient claims or guarantees”.

Biostimulants are obtained from natural or biological sources which improve the plant growth, efficiency of nutrients or nutrient uptake, and soil structure which ultimately improves plant response. The ‘First World Congress on Biostimulants in agriculture' which aimed to bring together scientists related to biostimulants' aspects in agriculture was held in Strasbourg in 2012 (Jardin 2015).

10.2 Main Categories of Biostimulants

10.2.1 Humic Acid

Humic substances (HS) are natural products developed form continuous degradation of different residues due to the metabolic activity of microorganisms present in soil. Humic substances are a mixture of substances like humins, humic acids, and fulvic acids based on molecular weight and solubilization of substance. Humic acids are easily dissolved in basic medium and precipitate in lower pH medium, while fulvic acids get easily solubilized in both acidic and alkali medium (Berbara and García 2014). Humin is a complex of humic and non-humic materials which are described as humic-containing substances (Rice and Mac Carthy 1990; Nardi et al. 2009). HS are isolated from the soils comprising proteins, biopolymers, lignins, and carbohydrate, which constitute the major role in microorganisms and plants (Kelleher and Simpson 2006; du Jardin 2012). Humic material results from the interaction among the decomposed material, plant roots, and microbes. The unevenness in the humic substances' effects arises due to the humic acid sources, the ecological situations, type of plant, dose, and mode of HS product (Eyheraguibel et al. 2008). Collapsing and oxidation of agricultural by-products by chemical reaction leads to the formation of humic-like compounds which could be utilized as alternative for natural humic substances (Rose et al. 2014).

Humic substances help in soil fertility improvement, including physical, chemical, biological, and physico-chemical properties of the soil. Humic substances' affects the root nutrition enrichment through different processes, reduce soil compaction, increase water retention, increase the nutrient uptake due to higher ion exchange activity of the soil involving polyanionic humic substances, and increase the phosphorus uptake via interaction of humic substances with calcium phosphate precipitation, affects the physiology of plants and composition of rhizosphere microorganisms (Berbara and García 2014; Varanini and Pinton 2001). Humic substances also affect the root nutrition through plasma membrane stimulation and converts free energy to trans-membrane electrochemical energy liberated during ATP hydrolysis. It contributes in cell wall loosening, enlargement, organ development, enhances respiration and invertase enzyme processes (Jindo et al. 2012a, b).

10.2.2 Protein Hydrolysates and Amino Acids

Protein hydrolysates consist of a combination of amino acids, denatured proteins, peptides, and polypeptides achieved through hydrolysis of proteins from plant and animal resources. Use of protein hydrolysate obtained from animal sources were identified as phytotoxic displaying adverse role on plant development as compared to plant origin. Chemical structure of PHs is affected by protein source and production process. Chemical protein hydrolysis of animal origin is generally preferred under acidic or alkaline conditions. Acid hydrolysis is a more hostile process, whereas alkaline hydrolysis is simple where proteins are easily solubilized through heating process with addition of alkaline agents. Chemical breakdown affect all peptide bonds leading to destruction of various amino acids and convert them into acidic forms with acid hydrolysis (García-Martínez et al. 2010). Another category includes individual amino acids-based protein products. For the synthesis of these proteins and non-protein amino acids, 20 structural amino acids are included predominantly identified in some plant species (Vranova et al. 2011).

Plant hydrolysates help to increase the performance of agricultural crops like plant biomass enhances the productivity, increase iron and nitrogen metabolism, and enhance nutrient and water uptake (Haplern et al. 2015). More nutrient uptake helps to increase soil microbial and enzymatic activity, improves nutrient mobility and solubility, and modifies the rhizosphere of plants (García-Martínez et al. 2010). Application of these structural and non-protein amino acids also offers protection against environmental stresses (Liang et al. 2013).

10.2.3 Inorganic Compounds

There are some chemical substances which are required for growth and development for some specific crop species. Al, Co, Na, Se, and Si are five major serviceable elements in soil and plants occurring as inorganic salts. These advantageous elements help in the strengthening of cell walls due to deposition of silica, which is expressed in specific environmental situations such as attack of pathogens and physiological stress. They also improve the quality of produce and provide tolerance against biotic and abiotic stresses, besides helping in cell wall rigidification, osmoregulation, reducing transpiration rate due to crystal deposition, heat regulation through reflection of rays, regulating enzymatic activity, antioxidant protection, synthesis of plant hormones, and protection against heavy metal toxicity (Pilon-Smits et al. 2009). Minerals of useful compounds such as phosphites, chlorides, carbonates, phosphates, and silicates are also included in this category and these can be applied as pesticide (Deliopoulos et al. 2010).

10.2.4 Chitosan and Other Biopolymers

Chitin is having a biopolymer named as chitosan in deacetylated form, produced organically and artificially. Polymers of controlled fluctuating sizes are very useful in nourishment, beautification, health, and in farming sectors. Polycationic elements have the ability to unite with broader range of cellular parts, including membranes of plasma, DNA, and cell membrane ingredients for the formation of chitosan oligomers, or bind with some specific receptors involved in defence by the regulation of genes (Hadwiger 2013; Jardin 2015). Chitosan and chitin seemingly use clear-cut receptors and signalling pathways. Chitosan binding results in cellular consequences such as Ca2+ leakage and deposition of hydrogen peroxide into the cell, causing major physiological changes. Scanning of proteins assure this assumption by studying plant tissues which are treated with chitosan (Ferri et al. 2014). Transpiration rates can be lowered by enhancing closing of stomata with the use of chitosan through an ABA-dependent mechanism. Many polymers of synthetic as well as biological origin are being used for increasing the agricultural productivity, providing resistance by activating the plant defence mechanism.

10.2.5 Microorganism

It is a well-known fact now that microorganism has immense potential in enhancing agricultural productivity (Hayat et al. 2010). Microbial inoculants are used in both government and non-government sector for research or evolution. Microbial inoculations are taken out from various macro and micro habitats such as soil material, waste from plants, water, and mulched material. Root exudates are produced by non-identical plant species or cultivars, which is in the favour of inoculated microorganism activity and also serves as biologically active substrates formed by the micro-organisms (Khalid et al. 2004). Soil inoculant's reproducibility has been checked in a wide span of soils or natural habitats. Commercial formulation is another alternative for inoculants' growth (Bashan et al. 2014). Only those microorganisms are useful for conventional agriculture that completely fit with inorganic compounds used for soil and the inorganic chemical used for the protection against various biotic stresses. Some fungi are heterogeneous in nature and show symbiotic behaviour. With the use of mycorrhiza, there is an increase in sustainable agriculture system because of the symbiotic benefits of mycorrhiza associated with nutrients, balancing of water and protection against stresses (Jardin 2015). Another category of microorganism is beneficial bacteria, showing interaction with plants in various ways. These bacterial associations may be temporary or permanent.

10.2.6 Fulvic Acid

Humic matters are fragmented in various forms such as humins, humic, and fulvic substances (Berbara and García 2014). Fulvic acid is different from humic acid because it contains more carboxyl groups, a higher number of total acidity and cation exchange capacity (Bocanegra et al. 2006). Chelation and mobilization of Fe and Al metal ions in soil is only possible due to fulvic acid (Calvo et al. 2014). The size of fulvic substances is smaller as compared to the humic substances, so it has an advantage in movement through micropores of biological and artificial membranes. Fulvic acid has combined capacity to chelate nutrients such as Fe and act as natural chelators and move through membranes and transport different nutrients (Bocanegra et al. 2006). Fulvic acid is able to enhance the capacity to show association with roots of plants (Varanini and Pinton 2001).

10.2.7 Seaweed Extract

To intensify soil fertility and soil productivity, seaweed was in use from many past years in pure form or may be as supplement in the form of composting (Craigie 2011). Seaweed extract act as chelators and as biostimulants for improving nutrient uptake and their utilization for improving soil properties and enhancing germination of seeds, improving growth, yield, and its related traits and also ameliorate shelf life (Mattner et al. 2013). The seaweed extract related to elements lower in mass acts as plant growth hormones and is provided in the form of biostimulants (Tarakhovskaya et al. 2007; Jardin 2015); on the other hand, when extracts have larger molecules like unique polysaccharides, polyphenols, and allelochemicals, they are used for resistance to stress in the form of biostimulants (González et al. 2013).

Mostly profitable extracts are generally obtained from seaweeds of brown algae, which include Ascophyllumnodosum, Fucus, Laminaria, Sargassum, and Turbinaria spp. (Sharma et al. 2012). Seaweed extract product is available in the market in the form of fluid or in dry form (Stephenson 1974), and this formulation is available in combined form of nutrients and fertilizers to enhance the growth of plants (Milton 1952; Craigie 2011).

Seaweeds are used as a source of organic matter in the agriculture from many last decades, but the biostimulant effect of seaweeds has been gaining in importance recently. Purified forms of seaweed extract generally contain various nutrients and enzymes and growth promoters for plant growth (Calvo et al. 2014). Seaweed's use for plant growth in different forms such as in the soil as the polysaccharides that contribute for water retention and soil aeration by forming gel, while on the other hand, directly on the plants in the form of foliar spray as polyanionic compounds which contribute for fixation and exchange of cations such as heavy metals (Craigie 2011).

10.3 Effects/Applications of Biostimulants in Different Crops

10.3.1 Plant Growth, Yield, and Nutrient Efficiency

In response to plant biostimulant, stimulation of plant growth has been associated with signalling molecules' activity in the plant metabolism. Enhanced growth and yield is also connected with higher nutrient uptake (Table 10.1). In maize, inoculation of *Bacillus* with AMF enhanced the plant growth with higher nutrient assimilation of total nitrogen, phosphorus, and potassium in plants (Wu et al. 2005). Sheng and He (2006) reported that application of *Bacillus edaphicus* enhanced K uptake due to the organic acids' synthesis which dissolve rock K ions. Use of PGPR exhibited significant nutrient uptake and higher plant dry weight in cotton and

Table 10.1 Effect of biostimulants application in different crops

Crop	Biostimulant	Effects of simulants	References
Coriandrum sativum L	Asahi SL (Sodium para-nitrophenolate)	Chilling stress, enhanced chlorophyll and carotenoids, decreased electrolyte leakage	Pokluda et al. (2016)
Solanum lycopersicum	Humic acid	Increased yield, total soluble solids, ascorbic acid	Yildrim (2007)
Solanum lycopersicum	Animal and plant derived PHs	Higher chlorophyll and iron concentration under saline soils	Cerdan et al. (2013)
Solanum lycopersicum	*Azospirillum brasilense*	Chilling stress, increased plant height and dry weight	Romero and Correa (2014)
Solanum lycopersicum	*Flavobacterium glaciei, Pseudomonas frederiksbergensis, Pseudomonas vancouverensis*	Increased proline content, decreased electrolyte leakage and lipid per oxidation	Subramanian et al. (2015, 2016)
Solanum lycopersicum	*Dunaliella salina* exo-polysaccharides	Salt stress, increased chlorophyll and protein content, decreased proline accumulation	Arroussi EL et al. (2018)
Solanum lycopersicum	*Arbuscular mycorrhizal* and *Pseudomonas fluorescens*	Enhanced fruit mass by 6.9 g	Bona et al. 2018
Solanum lycopersicum	*Ascophyllum nodosum*	Drought stress, increased plant growth, chlorophyll content, proline accumulation, and soluble sugars	Goñi et al. (2018)
Capsicum annuum	Humic acid	Increased shoot and root weights, nutrient content under saline conditions	Cimrin et al. 2010
Capsicum annuum	Fulvic acid	Higher carbohydrate, total phenolics, capsaicin, carotenoids, and antioxidant activity in fruit	Aminifard et al. 2012
Capsicum annuum	*Azospirillum brasilense/ Pantoea dispersa*	Salt stress, increased plant dry weight, plant growth rate, net assimilation rate, and CO_2 assimilation	Del-Amor and Cuadra-Crespo 2012
Brassica oleracea var. *italica*	*Ascophyllum nodosum* + amino acids	Drought stress and increased chlorophyll content	Kałuzewicz et al. (2017)
Cucurbita pepo	*Moringa* leaf extract	Drought stress, enhanced plant growth, soluble sugars, proline accumulation, chlorophyll content by 34.6%, decreased membrane stability and electrolyte leakage	Abd El-Mageed et al. (2017)

(continued)

Table 10.1 (continued)

Crop	Biostimulant	Effects of simulants	References
Phaseolus vulgaris	Humic acid and Fulvic acid extracted from charcoal mine	Higher nutrient uptake by 41%	Rosa et al. (2009)
Phaseolus vulgaris	Humic acid	Salt stress, increased nutrient uptake, proline content root and shoot dry weight, reduced soil electrical conductivity (EC)	Aydin et al. (2012)
Phaseolus vulgaris	*Licorice* root extract	Salt stress, higher growth, yield, chlorophyll content, free proline accumulation, carbohydrates, and soluble sugars	Rady et al. (2013)
Lactuca sativa	Protein hydrolysates	Salt stress, increased yield, dry biomass, chlorophyll content, plant nitrogen metabolism, osmolytes, glucosynolates, nutrient uptake and reduced oxidative stress	Lucini et al. (2015)
Lactuca sativa	Retrosal	Salt stress, increased fresh weight, chlorophyll accumulation, gas exchange, reduced proline content, and ABA	Bulgari et al. 2019
Cicer arietinum	*Sargassum muticum* and *Jania rubens*	Salt stress, increased growth, chlorophyll, carotenoid, soluble sugars, and phenol content	Abdel Late et al. (2017)
Cucumis sativus	Humic acid	Enhanced yield and sugar content	Karakurt et al. (2009)
Cucumis sativus	Humic acid	Increased shoot growth, NO_3 activity	Mora et al. (2010)
Cucumis sativus	Humic acid	Increased growth, yield, and nutrient efficiency	El-Nemr et al. (2012)
Glycine max	Humic acid	Increase in chlorophyll content	Befrozfar et al. (2013)
Abelmoschus esculentus	Humic acids	Increased fruit yield	Kirn et al. 2010
Solanum tuberosum	Humic acid	Enhanced yield, protein content, and chlorophyll accumulation	Selim et al. (2012)
Triticum aestivum	Fulvic acid	Enhancement of glutamic oxaloacetic transaminase (GOT) enzyme	Gu et al. (2001)
Triticum aestivum	Fulvic acid	Enhanced seedling root growth, proline accumulation, lowers Se toxicity and membrane permeability	Peng et al. (2001)

(continued)

Table 10.1 (continued)

Crop	Biostimulant	Effects of simulants	References
Triticum aestivum	Humic acid	Higher biomass, plant height, and nutrient uptake	Tahir et al. (2011)
Zea mays	Humic acid	Improved root development, root length	Canellas et al. (2009)
Zea mays	Humic acid	Enhanced root growth	Asli and Neumann (2010)
Zea mays	Humic substances	Increased phenyl propanoid pathway, amino acids; lowers phenylalanine and tyrosine accumulation	Schiavon et al. (2010)
Zea mays	Fulvic acid	Increased leaf area, plant dry weight, chlorophyll, yield, CO_2 assimilation rate, and proline concentration	Anjum et al. (2011)
Zea mays	Salicylic acid–chitosan	Higher chlorophyll content in the maize leaves	Kumaraswamy et al. (2019)
Oryza sativa	Fulvic acid	Higher Fe uptake	Pandeya et al. (1998)
Oryza sativa	Humic acid	Enhanced growth of plants, proline content, reduce oxidative stress, and helps to maintain membrane permeability under drought stress	Garcia et al. (2012)
Oryza sativa	Humic acid	Maintained peroxidase activity under drought conditions; reduced lipid peroxidation	García et al. (2013)
Vicia faba	Fulvic acid	Higher Pb uptake; reduced lipid peroxidation	Shahid et al. (2012)
Chrysanthemum	Humic acid	Increased flower diameter (33%), biomass accumulation	Fan et al. (2014)
Allium sativum	Biofertilizer (*Azobacter, Azospirillum* and *Klebsiella*)	Enhanced bulb yield (from 2 to 6%), quality and storage properties of bulb	Abdel-Razzak and El-Sharkawy (2013)
Allium sativum	Humic acid	Enhanced mineral nutrition efficiency and pyruvic acid which increased pungency	Denre et al. 2014
Allium cepa	Bee-honey based biostimulant	Increased biomass, yield, photosynthetic pigment accumulation, osmoprotectants, membrane stability, enzymatic and non-enzymatic antioxidant	Semida et al. (2019)

wheat (Egamberdiyeva and Höflich 2004; Shaharoona et al. 2008). In lettuce, combination of both *Pseudomonas* and the AMF *Glomus* enhanced nutrient uptake like iron, calcium, and manganese (Kohler et al. 2008). Use of siderophore producing *Streptomyces* strains significantly increased growth parameters in tomato and rice (Verma et al. 2011).

Humic materials elicit different morphological changes, which ultimately affect growth of plants (Clapp et al. 2001; Nardi et al. 2009). A significant increase in seedling root growth and enhancement of lateral roots has been reported by various scientists in different crops like wheat (Peng et al. 2001; Tahir et al. 2011), maize (Canellas et al. 2009; Jindo et al. 2012a, b), Arabidopsis (Dobbss et al. 2010), tomato (Canellas et al. 2011), pepper (Cimrin et al. 2010) and *Lantana camara* (Costa et al. 2008). Higher shoot growth development was reported in wheat (Tahir et al. 2011), maize (Eyheraguibel et al. 2008), tomato (Adani et al. 1998), cucumber (Mora et al. 2010), and pepper (Cimrin et al. 2010). Multiple use of humic acid in soil inhibited the shoot growth of maize grown under hydroponic system (Asli and Neumann 2010). In okra, application of three different doses of humic acid increased the fruits per plant and ultimately yield was increased (Kirn et al. 2010). With the humic acid, quality of wine was improved in grapes due to increase in nitrogen content of grapes (Morard et al. 2011). Karakurt et al. (2009) reported significantly higher total yield, weight of fruit, reducing sugars, total soluble sugars (TSS) and chlorophyll activity in pepper grown in Turkey with foliar spray of humic acid. Similarly, Yildrim (2007) assessed the effects of humic acid and identified increase in early and total yield with higher TSS and ascorbic acid in tomato. In Arabidopsis and micro-Tom tomato, fulvic acid improved the length of lateral roots (Dobbss et al. 2007). With the application of fulvic acid, shoot growth was enhanced in tomato (Lulakis and Petsas 1995), plant biomass in wheat and maize (Anjum et al. 2011), and more number of flowers in cucumber was reported (Rauthan and Schnitzer 1981). Application of humic acid with 90.7% fulvic acid improved the nutrient uptake in plants (Sánchez-Sánchez et al. 2002). In sunflower, Bocanegra et al. (2006) identified that fulvic acid chelated Fe^{3+} and enhanced availability of iron with Hoagland solution containing ^{59}Fe. Foliar spray with Siapton increased yield by 12% in papaya (Morales-Payan and Stall 2003). Increase in height and more flowers number/plant was reported in tomato fertilized with Siapton. Use of a protein hydrolysate increased fruit yield, number, and weight in tomato (Koukounararas et al. 2013).

Seaweed extract application improved root development in a number of species comprising Arabidopsis (Rayorath et al. 2008), maize (Jeannin et al. 1991), winter rapeseed (Jannin et al. 2013), strawberry (Alam et al. 2013), grape (Mugnai et al. 2008), and lodgepole pine (MacDonald et al. 2012). Combination of growth regulators with seaweed extract increased lateral root formation, total root volume, and root length (Vernieri et al. 2005; Zodape et al. 2011; Khan et al. 2011a, b). Improvement in formation of roots (Vernieri et al. 2005), root length, and volume (Mancuso et al. 2006; Zodape et al. 2011) have been identified with application of plant growth regulators with seaweed extracts (Khan et al. 2011a, b). Foliar application of seaweed extract enhanced nutrient content and increased macro and micro nutrients in soybean (Zodape et al. 2011), winter rapeseed (Jannin et al. 2013), tomato (Zodape et al. 2011), lettuce (Crouch et al. 1990), and grape (Mancuso et al. 2006). Microorganisms' diversity and rhizosphere activity was increased in strawberry with seaweed extract as biostimulant, while plant growth and root nodulation was stimulated in alfalfa (Khan et al. 2012, 2013). Colla et al. (2014) identified positive effects of Protein hydrolysates (PHs) on plant growth, nutrition, elongated the coleoptile, increased shoot, root biomass, and root length in corn.

Pseudomonas psychrotolerans strain enhanced growth and nitrogen fixation in rice (Liu et al. 2017). Organic biostimulants, including vermicompost, stone dust, organic herbs, and malt sprouts exhibited positive effects on growth of plants by altering the microbiota on the above-ground parts of a plant (Mahnert et al. 2018). Wilson et al. (2018) identified that gelatin hydrolysate application in cucumber boosted genes activity responsible for synthesis of amino acid permeases and carriers of amino acids and nitrogen. Gelatin hydrolysate acted as a source of nitrogen. Luziatelli et al. (2019) observed the effect of different stimulant combinations on lettuce growth. Shoot fresh weight was higher as compared to control and increased the growth of epiphytic bacteria and ultimately enhanced the productivity of lettuce. Cozzolino et al. (2021) reported the effect of three biostimulants (brown seaweed, protein hydrolysate and a tropical plant extract) in tomato. Protein hydrolysates and seaweed extract increased the marketable yield by 18.3%, TSS, hydrophilic antioxidant activity, and ascorbic acid and boosted the nitrogen uptake efficiency.

10.3.2 Physiology and Metabolism

Different categories show different effects on plant metabolism and physiology of plants. Humic material shows favourable ramification on morphology of plants because it enhances properties of soil by improving fertility of soil, texture, or structure and enhances uptake capacity of nutrients and biomass of the roots is improved in the plants (Table 10.1) (Trevisan et al. 2010). A fragment of humus makes direct association with architecture of roots. Humic acids correlated with organelles of cells just for the initial short time (Berbara and García 2014). Direct uptake of humic matter shows direct effect on physiology of plants (Nardi et al. 2009). ATPase activity is increased due to humus substances in the cells of roots and enhances surface of roots, root density also increases (Canellas et al. 2009). In favour of above, Calvo et al. (2014) explains the activity of some promoters mainly of DR5 which is related to the production of auxins and combined with a gene named as GUS in genetically modified tomato. H^+-ATPase action, act as the activity of protons. Activity of metabolites and other ions enhances throughout the membrane and increases the level of energy because of H^+-ATPase with the help of electrochemical gradient. Due to increase in activity of H+ -ATPase, extension and development of roots increases in maize (Canellas et al. 2009). Further effects of humic acids studied by gene interaction and their metabolites, which identified by using protemotics, trancriptomics, and microarray analysis.

Fulvic acids affect plant physiology, which is explained by development of plants and resistance against different stresses. Development of roots enhanced by using fulvic acid in small hydoponic system with large-Tom tomatoes, dgtwas (diageotropica gene of tomato) mutant is mainly used because these are insensitive to the auxin used in this research. Photosynthetic activity, rate of the transpiration rate or the CO_2 concentration level in the intercellular space of the cells is increased with fulvic acid that ultimately concerned with the development of crops (Calvo et al. 2014). Deposition of the stress hormone named as proline increases due to Fulvic substances therapy in water logged or good-watered crops (Peng et al. 2001). Protein hydrolysates enhance metabolism and also assimilation of nitrogen in plants. In maize, application of Siapton increases function of NAD-dependent glutamate dehydrogenase, nitrate reductase, and malate dehydrogenase. In alfalfa hydrolyzation of amino acids put into maize, i.e. grown in glasshouse with hydroponic conditions, this situation enhances the concentration of the main enzymes involved in the process or tricarboxylic acid cycle and also other enzymes which show involvement in the assimilation and the reduction processes of nitrogen. Expression of the main three enzymes in the TCA cycle increased, which was assured by the reverse transcriptase polymerase chain reaction. Plant metabolism of plants are adversely affected by seaweed extracts. Proteins i.e. solubility of proteins in natural form have antioxidant properties, phenolic or flavonoid glad in *Spinacia oleraceae* enhanced after treating with extracts of algae mainly brown (Fan et al. 2013). Nitrogen metabolism is increased because of transcriptional enzymes, antioxidative ability, and glycine betaine synthesis. Protein coding some genes that are involved in the fixation of carbon compounds with the help of Rubisco or carbonic anhydrase, which was increased by withdrawal of some algaes and increased content of starch by promoting its synthesis, which enhances starch synthesis. NRT1.1 gene have important role in feel of transport for auxin that ultimately shows involvement for nitrate transporters, (Castaings et al. 2011), because of this growth of roots increases.

10.3.3 Antioxidant Properties

In today's research, the antioxidant properties of fruits and vegetables are gaining wide importance because these properties have the ability to stop the division of tumour-causing cells and also provide protection against stresses caused by free radicals. These stresses generally cause damage to the genetic material, organelles of cells. The use of biostimulants in crop improvement affects the action of various enzymes that influence the fruits and vegetables' antioxidant properties. Crops having lycopene, ascorbic acid, and phenols had these properties.

10.3.4 Stimulation of Plant Defences Against Abiotic Stresses

Huge losses in yield have been reported due to abiotic stress in agriculture due to climatic conditions, which also leads to degradation of soil. Applications of biostimulants like microbial inoculants, fulvic and humic acids, and protein hydro show positive response on growth of plants and protects from various environmental stresses, thus lowering yield losses. Rhizobium and Azospirillum inoculation imparts salt tolerance. Inoculation of A. lipoferum eliminated the adverse effects due to salinity in wheat (Bacilio et al. 2004). Use of A. brasilense eliminated the symptoms of water stress in maize under protected conditions (Casanovas et al. 2002). Inoculation of P. putida and B. megaterium with plant growth regulators improved plant biomass and moisture content in white clover under dry conditions (Marulanda et al. 2009). Mycorrhizal symbioses increased drought tolerance in wheat, corn, soybean, lettuce, and onion (Brundrett 1991a, b; Augé 2001; Calvo et al. 2014). Application of various microbial inoculants mitigated the drought stress in wheat, barley, tomato, pepper, and ryegrass (Bae et al. 2009; Singh et al. 2011). Karlidag et al. (2013) identified that inoculation of *Bacillus* species and *Staphylococcus* improved chlorophyll, nutrient uptake and yield in saline conditions in strawberry. Inoculation of *Azospirillum lipoferum* improved plant biomass, enhanced bacterial activity of ACC-deaminase, which lowered the ethylene concentration caused by salinity in canola (Baniaghil et al. 2013).

Application of humic acid reduced electrical conductivity of soil and leakage of proline content from plants provides salt tolerance under saline conditions (Aydin et al. 2012). It has also eliminated the adverse effects on growth and flowering stage under saline conditions in chrysanthemum (Mazhar et al. 2012). Humic materials enhanced the growth, decreased the proline synthesis and ABA level in pistachio in saline water (Moghaddam and Soleimani 2012). It also enhanced the plant biomass, germination, carotenoids and carbohydrates under moisture stress conditions due to higher photosynthetic activity (Garcia et al. 2012). In wheat, combination of both fulvic acid with low Selenium content enhanced root growth and decreased the symptoms of stunting; chlorosis and Ca^{2+} uptake was higher (Peng et al. 2001). Harper et al. (1995) identified that fulvic acid eliminated the adverse effects of Aluminium on root development in maize. Foliar application of fulvic acid enhanced the accumulation of rare earth elements (REEs) (La3+, Gd3+, Y3) which could reduce their build-up in soil in wheat (Murillo et al. 2005).

Animal membrane hydrolysate and Macro-Sorb increased photochemical efficiency and cell integrity in perennial grass when exposed to high temperature (Kauffman III et al. 2007). In Arabidopsis, Apone et al. (2010) observed that amino acid mixture obtained from plant membrane enhanced the response of three stress genes with two other genes involved in the reactive oxygen species. Use of alfalfa hydrolysate enhanced plant dry weight, lowered the antioxidant and enzymatic activity and phenolic compound production in maize grown under saline conditions (Ertani et al. 2013). Glycine betaine and proline works as osmoregulants, stabilizing protein and enzymes activity and protects the plant cell membranes from denaturation due to higher salt activity and temperature. Glycine betaine and proline accumulation is associated with stress tolerance, and exogenous application increases abiotic stress tolerance in cereal crops (Chen and Murata 2008; dos Reis et al. 2012; Ahmad et al. 2013). Glutamate and ornithine enhances salt tolerance in plants (da Rocha et al. 2012). Extracts of A. Nodosum increased freezing tolerance in Arabidopsis and protect the membrane integrity, decrease the activity of chlorophyllase genes and enhance the expression of three cold tolerance genes (Rayirath et al. 2009).

Response of plants against different abiotic stress has been studied using different molecular techniques. Plant metabolism may be changed due to the production of regulatory molecules like salicylic acid, ABA; proline, which stabilizes protein and enzymatic activity, helps to sustain cell turgidity and eliminates reactive oxygen species. Nair et al. (2012) determined that 1113 genes expressed in the lipophilic components (LPC) in seaweed extract treated plants showing freezing stress and 463 genes were upregulated linked with stress responses, enhanced sugar and lipid production and response to ABA in Arabidopsis using genomic approaches. Treatment with *A. nodosum* enhanced the expression of salt tolerance genes and suppressed the other genes in Arabidopsis undergoing salt stress (Jithesh et al. 2012).

10.3.5 Effect of Biostimulants on Physical Characteristics

Biostimulant's application alters the physical and mechanical properties like firmness, shelf life, cell wall flexibility, etc. Biostimulants with chitosan and phenolic compounds resulted in loss of firmness of fruits in raspberry, while not affected with titanium compounds (Grajkowski and Ochmian 2007). In chilli pepper, the application of protein hydrolysates derived from alfalfa and red grapes enhanced capsaicin in red chilli fruit, while increasing the concentration of chlorogenic acid, p-hydroxybenzoic acid, and p-coumaric acid in green fruits (Ertani et al. 2014).

Cracking was decreased with application of spic cytozyme in pomegranate (Aziz et al. 2013). Seaweed extract enhanced the water content and surface area of the leaves. Cell turgidity, surface area and photosynthesis activity were improved in spinach under stress conditions (Xu and Leskovar 2015). Biostimulant application leads to stiffening of cell wall and lowers its extension (Tarantino et al. 2018). Biostimulants increase the flexibility of cell walls and prolong the shelf-life of fruits to maintain proper size and shape. Biopolymers of polysaccharides, humic and fulvic acids increased the mechanical strength of apricot fruits. Biostimulants also alter the fruit shape and colour. Consumers prefer larger fruit with more length and diameter with proper colour development (Tarantino et al. 2018). Presence of anthocyanin influences the colour of the fruit. Biostimulant application developed a lighter skin colour of apricot fruits in first year and red colour skin in second year (Tarantino et al. 2018). Application of glycine and betaine in cherries developed darker skin colour, which is caused due to higher content of antioxidants.

10.3.6 Effect on Chemical Composition

Application of biostimulants alters the chemical properties of fruits, which include vitamins, acidity, dry weight or biomass and soluble solids. Biostimulants with phenol content or chitosan decreased the dissolved solids content in raspberry fruits, whereas content of dissolved solids in fruits increased with biostimulants containing titanium compounds (Grajkowski and Ochmian 2007). Humic and fulvic substances, carboxylic acids, and polysaccharides application improved the taste of fruits by increasing the value of SSC (10.7°Brix) in apricots (Tarantino et al. 2018). Phenolic and titanium material increased the fruit acidity in raspberry (Grajkowski and Ochmian 2007). Similarly, fruit acidity was reduced with application of humic compounds, polysaccharides and carboxylic acids were determined in apricot (Tarantino et al. 2018). Biostimulants containing phenolic, chitosan, and titanium compounds enhanced the Vitamin C and nitrate level in raspberries, while Vitamin C was boosted with the phenolic compounds (Grajkowski and Ochmian 2007). Use of toasted and non-toasted biostimulants augmented the phenol concentration, hydroxycinnamic acids, which ultimately affect the taste and quality of grapevine (Sánchez-Gómez et al. 2017). *A. nodosum* at 1.5 and 3 kg/ha enhanced the phenol compounds in grapes (Frioni et al. 2018). Salicylic acid-chitosan nanoparticles enhanced the chlorophyll and photosynthetic activity in leek corn (Fawzy 2012). Use of leaf extract from *Moringa oleifera* boosted chlorophyll in *Cucurbita pepo* leaves (Abd El-Mageed et al. 2017).

Seaweed extract *A. nodosum* and a silicon extract application increased the sugar level in strawberry (Weber et al. 2018). Extract derived from *M. oleifera* leaves enhanced total soluble sugar (TSS) content by 80.6% in pumpkin (Abd El-Mageed et al. 2017). Arbuscular mycorrhizal and *Pseudomonas* enhanced increased Vitamin C and β-carotene in tomato (Bona et al. 2018). Use of biostimulant with sewage sludge increased protein content in maize by 30% (Tejada et al. 2016). Use of biostimulant extracted from pressing olive oil process enhanced 19% protein in maize (Alberola et al. 2008).

10.4 Methods of Application

Commonly biostimulants are available in ready-to-use material to prepare a liquid solution. These can be directly added to soil in the form of soil preparations or as foliar application (Kocira et al. 2018). Biostimulants having humic and nitrogen compounds are directly applied into the soil in the form of granules affecting the structure of the root, increasing ability to absorb nutrients. The plant extracts and seaweed types are applied as foliar sprays. Alternatively, to reduce labour cost, biostimulants can also be applied by integrating in the irrigation system. Time of application of biostimulants is also very wide. These can be used regularly during the entire growth period at certain intervals or at one time during deficiency of nutrients in the plants. When applied upon the appearance of deficiency symptoms, it has been observed that foliar application is more effective than the soil application. Foliar applications of biostimulants safeguard the plant against various stresses. It could also be applied in biomass form extracted from seaweed, particularly in areas situated near the source of seaweed acquisition. The best time for application is at the time of stomata opening in morning hours and the assimilation rate is higher (Goñi et al. 2018). Application of biostimulants directly on harvested fruits (Battacharyya et al. 2015) has been observed to extend the shelf-life storage life of oranges significantly.

10.5 Conclusion

Plant biostimulants are going to play an important role by making a significant contribution to ecologically and economically sustainable crop production systems in the coming years. The use of biostimulants will reduce the amount of mineral fertilizers, thus reducing soil, water, and air pollution. It is a well-established fact that biostimulant application has positive impact on crop quality and performance without affecting soil and environment health. The demand for the biostimulants is increasing day by day; and it is estimated that global biostimulant market will grow to almost 5 billion dollars by 2025 from 2.6 billion dollars in 2019 at a compound annual growth rate of 11.2%. The Indian biostimulant market is projected to witness a CAGR of 16.49% to reach a total market size of US$180.949 million by 2023, increasing from US$71.232 million in 2017. However, there is a dire need to understand the nature and their positive effects, making them a hot topic of research. Presently, plant biostimulants are only considered in a category of complementary inputs applied along with the chemical fertilizers. With the aid of molecular and physiological tools, the biostimulants can be employed more effectively to enhance crop productivity.

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