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Diversity of Plant Biostimulants in Plant Growth Promotion and Stress Protection in Crop and Fibrous Plants

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Abstract
Nowadays, farmers and entrepreneurs strive to obtain higher and better quality seeds and plant products containing fibre by providing plants with optimal growth conditions using agrotechnical methods such as crop rotation, enhancing soil quality and protection against diseases. The use of biostimulants, substances that promote plant growth and resistance, seems to be the best way to achieve satisfying results. Biostimulants are included in the modern plant industry and environment-friendly crop management as they enhance the quality of crops while reducing chemical inputs. In textile plants, biostimulants can affect fibre structures regardless of the part of the plant they come from – seed, bast or leaf. The possible positive influence may be related to the increase in fibre length, shape, diameter, strength, flexibility, abrasion resistance, moisture absorbency, and antimicrobial properties. The purpose of this review is to better understand the unique characteristics of different biostimulants, which have a great influence on crop and fibrous plant properties.

Key words: *biostimulants, natural fibre, plant protection, stress factors, mode of action.*

The wide range of biostimulant components used in crop and fibrous plant cultivation (definitions and classification)

Nowadays, a lot of attention is paid to plant production technologies for crop improvement that encounter restrictions due to the inability to use the biological potential inherent in the cultivar [1-3]. Hence, the constant search for new solutions aims to provide plants with the most favorable conditions for growth and development, even by limiting various biotic and abiotic stresses, and ultimately to increase yield [1, 4, 5]. For example, the use of environmentally-friendly substances, for example biostimulants, which can both directly and indirectly affect plants, can impact the metabolism of plants, thereby improving the efficiency of nutrients, root growth, and thus increase yields [6]. The use of more sustainable methods in agriculture production is caused by the growing demand for food, feed, fuel, fibre, and raw materials, as well as by the increasing resource depletion and ecosystem degradation [7]. The European Biostimulants Industry Council (EBIC) presented a definition of plant biostimulants: "substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality. Biostimulants have no direct action against pests, and therefore do not fall within the regulatory framework of pesticides" [8, 9]. Biostimulants are used at all stages of agricultural production, including seed treatment and foliar spraying during plant growth and harvesting [1]. The biostimulant can activate the N metabolism, release P from soil, stimulate soil microbial activity and root growth, and induce better plant formation [1, 10]. Moreover, biostimulants can also mitigate the negative impact of abiotic stress factors on plants, and thus they control drought, heat, salinity, oxidative stress and mechanical damage [1, 2, 11]. Biostimulants can also participate in breaking dormancy, stimulate plant growth and development, increase fruit size, promote the root system, and increase the activities of photosynthetic and other vegetative tissues [12].

Biostimulants can be classified as follows: humic substances, amino acids and other nitrogenated compounds, inorganic compounds, seaweed extracts and botanicals, chitin and other biopolymers, and other beneficial elements (fungi and bacteria) [13]. Among the many review papers on specific biostimulants available, they have widely studied protein hydrolysates, seaweed extracts, humic and fulvic acids, and biological control agents (BCAs), including *Trichoderma* [14, 15]. ***Table 1*** shows selected reports on the biostimulant influence on horticultural crops and fibrous plants.

The biological activity of seaweed and other algal biomass may be very important in plant cultures, where they are exploited as organic soil amendments to enhance soil fertility and crop productivity [33-35]. Moreover, seaweed extracts affect seed germination and establishment, plant growth, yield, flower setting and fruit production, resistance to biotic and abiotic stresses, and the postharvest shelf life [3].

Humic substances (HS) are natural components of soil organic matter, originating not only from plant, animal and microbial residue decomposition but also from the metabolic activity of soil microbes. Among heterogeneous compounds there are humins, humic acids and fulvic acids, divided based on their molecular weight and solubility [13]. Because of the occurrance of oxygen, nitrogen, and sulfur in the structure containing functional groups, humic substances can participate in forming stable complexes with metal microelements. It can influence the retention of micronutrients by changing the pH of the solution [36, 37]. The application of partially wetted organic waste obtained from plants, wood, food and other human activities, beneficial to the soil, can support recycling. Thus, understanding the biological HS mechanism through its function is becoming an important tool in solving environmental problems [38].

Microorganisms, including fungi which belong to *Trichoderma* spp., such as *Trichoderma atroviride* [26, 39], *Trichoderma virens* [40, 41], *Trichoderma harzianum* [42, 43], *Trichoderma longibrachiatum* [44], *Trichoderma hamatum* [45] and *Trichoderma asperellum* [46], are an important group of plant biostimulants.

***Table 1.** Selected reports of biostimulant effects on horticultural crops and fibrous plants.*

Biostimulant	Plant	Stress and effects	References
Seaweed extract	Arabidopsis	Cold tolerance	Rayirath et al. [16]
Seaweed extract	Maize	Cold tolerance	Bradáčová et al. [17]
Seaweed extract	Spirea	Drought tolerance	Elansary et al. [18]
Seaweed and humic acid	Bentgrass	Drought tolerance	Zhang et al. [19]
Humic acid	Rice	Oxidative and drought tolerance	García et al. [20]
Humic acid	Cucumber	Increased plant growth and yield	El-Nemr et al. [21]
Fulvic acid	Maize	Increased chlorophyll content	Anjum et al. [22]
Fulvic acid	Wheat	Enhanced seedling root growth	Peng et al. [23]
Humic acid	Cotton	Salinity stress tolerance	Rady et al. [24]
Humic and fulvic acids	Flax	Promoted growth and development	Belopukhov et al. [25]
Trichoderma atroviride MUCL 45632	Pepper	Enhanced shoot and Root dry weight	Colla et al. [26]
Trichoderma harzianum	Tomato	Promoted shoot and root growth	Azarmi et al. [27]
Trichoderma atroviride	Cucumber	*Rhizoctonia solani*	Nawrocka et al. [28]
Protein hydrolysate	Maize	Salt tolerance	Ertani et al. [29]
Protein hydrolysate	Wheat	Heavy metal tolerance	Zhu et al. [30]
Protein hydrolysate	Lettuce	Salt tolerance	Lucini et. al. [31]
Protein hydrolysate	Tomato	Increased root and shoot growth	Colla et al. [7]
Protein hydrolysate	Grapevine	*Plasmopara viticola*	Lachbab et al. [32]

These microorganisms are the most common saprophytic fungi in the ecosphere, characterised by rapid growth and the intensive production of spores under different environmental conditions, including changing temperature, nutrient status, and pH, which allows them to effectively colonise plant roots and shoots [15, 39, 47, 48]. Simultaneously, *Trichoderma* are one of the most used microbiological components of biopreparations applied in ecofriendly white biotechnology [49-51]. Members of the genus *Trichoderma* are used alone, in consortia with other fungi or bacteria as well as with organic and inorganic substances in multi-component soil and foliar biopreparations. On the market, *Trichoderma* spp. are available in the form of powders, granules on the base of an organic carrier, or solutions. Depending on the form, the media may include either fungi hyphae and spores or isolated elicitors and secondary metabolites responsible for the biostimulatory effect [39, 49, 52, 51].

Among protein-based biostimulants there are two main groups. In the first there are protein hydrolysates (PHs), including a mixture of peptides and amino acids of animal or plant origin. The second group consists of individual amino acids (glutamate, glutamine, proline, glycine and betaine) [3, 53]. There are a few methods of protein hydrolysate preparation: enzymatic, chemical or thermal hydrolysis of a variety of animal and plant residues [3]. Among the second category of protein-based biostimulants there are twenty individual structural amino acids which participate in the synthesis of proteins and non-protein amino acids [3, 54]. Applied exogenously, amino acids can affect biological processes acting directly as signal molecules or affecting plant hormones [1, 55]. According to Kauffman et al. [56], amino acid-based biostimulants are easily absorbed and displaced by plant tissues, and after absorbtion they have the ability to act as compatible osmolytes, transport regulators, signaling molecules, and crack opening modulators, and they can detoxify heavy metals.

Effect of biostimulants on plant growth and development

Seaweed extracts are used in agriculture as soil conditioners or plant stimulants [2]. According to many studies [2, 45, 57, 58], seaweed extracts are used to spray leaves in order to increase plant growth, cold, drought and salt tolerance, photosynthetic activity and resistance to fungi, bacteria and viruses, thus improving the efficiency and productivity of many crops. The seaweeds used to produce biostimulants contain cytokinins and auxins (IAA) or other hormone-like substances which, similar to the registered plant growth regulators, may act as hormones stimulating plant growth [2, 59].

Numerous studies have shown that HS not only enhance root, leaf and shoot growth but also stimulate the germination of various crop species [38]. These positive effects are related to the interaction between HS and physiological and metabolic processes. HS stimulate nutrient uptake and cell permeability, and appear to regulate the mechanisms involved in stimulating plant growth, through the induction of a carbon and nitrogen metabolism [60]. The kind of HS effects on plant growth and stress resistance in plants depends on their origin, molecular size and chemical characteristics [36]. HS can stimulate plant growth by improving the absorption of nutrients through releasing hormone-like effectors such as IAA [2, 61]. Canellas et al. [62] and Nardi et al. [63] observed that plants treated with HS of different origin were able to induce the proliferation of lateral roots and root hairs, which could be related to the activation of signaling pathways of phytohormones, especially auxin, nitric oxide, Ca^{2+} and reactive oxygen species (ROS) [36, 38, 64-68]. HS also stimulate shoot elongation and an increase in the accumulation of leaf nutrients and chlorophyll biosynthesis [2, 69, 70]. HS enhance the uptake of macro- and micronutrients, due to the increased cation exchange capacity of the soil [13]. A positive effect of HS on nutrient uptake was reported for major inorganic elements, such as nitrogen, phosphorus, potassium and sulphur; however, different HS fractions seem to differently affect their uptake [38]. The hormonal effect of HS is related to containing functional groups recognised by the reception/signalling complexes of plant hormonal pathways [13].

The positive effects of microorganism-based biostimulants are mainly related to the promotion of seed germination, plant growth and development, management of different phytopathogens, and the increase in the quality of plants used in industry [39, 47, 48]. *Trichoderma* may stimulate plant growth by increasing the availability, uptake and transport of biogenic elements, including N, P and other nutrients from the soil to the plant, as well as by producing compounds which mimic phytohormones accelerating seed germination and plant growth [48, 71]. For example, growth promotion by phytohormones containing IAA was observed in plants treated with *T. virens* [72] and by gibberellic acid (GA_3) in plants treated with *T. harzianum* [73]. As a consequence, *Trichoderma* may positively affect vegetable, fruit, cereal and fibre crops by enhancement of the yield rate and quality as well as the crop production standard. For example, the positive influence of biostimulants based on *T. atroviride* on the growth and yield of zucchini was proven by Colla et al. [74], whereas in the study of Velmourougane et al. [75], *Trichoderma–Azotobacter* biofilm inoculation improved soil nutrient availability and plant growth in wheat and cotton. Additionally, *Trichoderma viride* was shown to significantly increase the growth of genetically modified Bt cotton (*Bacillus thuringiensis*) [76].

Various amino acids and peptides act as signal molecules in the regulation of plant growth and development [1]. Peptide signaling is important in various aspects of plant development and growth regulation, including meristem organisation, leaf morphogenesis and defense responses to biotic and abiotic stress [1, 53]. Amino acids and small peptides are absorbed by both roots and leaves and then transported within the plant [74]. However, the root availability of amino acids and peptides can be strongly reduced by soil microbial activity [77]. Some of the experimental studies conducted that tested the effects of PH under both field and controlled conditions showed that they increased shoot and root biomass and stimulated the productivity of several crops such as corn, kiwi, lettuce, lily, papaya, passion fruit, pepper and tomato; moreover, they stimulated the N metabolism and assimilation [78]. Nitrogen is an essential macroelement whose availability in soil plays a key role in plant growth and development and crop yield [79]. Nitrate (NO_3^-) and ammonium (NH_4^+) are N forms preferred by plants, but they are in short supply in most ecosystems as well as in agricultural lands [77]. Miller et al. [80] and Fan et al. [81] showed that amino acids (especially glutamine and arginine) played a signalling role in the regulation of the N uptake by roots. Moreover, PH effectively improved the activity of enzymes which participate in the N and C metabolism [29, 74]. Similarly, the application of plant-derived PH (Trainer) infuenced N assimilation in corn seedlings grown under controlled environmental conditions [74, 82].

Biostimulants involved in the induction of plant resistance to abiotic and biotic stresses

Seaweed extracts are emerging as commercial formulations for use as plant growth – promoting factors and as a method to improve tolerance to salinity, heat, and drought [83]. The pretreatment of tall fescue (*Festuca arundinacea* Schreb.) and creeping bentgrass (*Agrostis palustris* Huds. A.) with seaweed extract and humic acid increased leaf hydration under dry soil conditions, root and shoot growth as well as the antioxidant capacity [83]. Santaniello et al. [84] investigated the effects of *Ascophyllum nodosum* extract (ANE) on the regulation of water stress responses in *Arabidopsis* plants, in terms of both photosynthesis performance and the impact on gene expression. The researchers suggested that drought stressed plants treated with ANE were able to maintain strong stomatal control and relatively high values of both water use efficiency (WUE) and mesophyll conductance during the last phase of dehydration. Thus, pretreatment with ANE can effectively acclimate plants to the incoming stress, promoting increased WUE and dehydration tolerance.

Besides the significant changes in the plant primary metabolism and nutrient uptake, HS may also strongly influence the secondary metabolism [53]. For example, Olivares et al. [85] observed that HS enhanced the expression of phenylalanine (tyrosine) ammonialyase (PAL/TAL), which catalyses the first major stage of phenol biosynthesis by converting phenylalanine into trans-cinnamic acid and tyrosine to p-coumaric acid. This stimulating effect of HS on the secondary plant metabolism provides an innovative approach to plant exploration stress responses [60]. Anjum et al. [22] showed that the treatment of maize with fulvic acid caused an increase in the photosynthesis, transpiration rate and intercellular CO_2 concentration, which are associated with plant growth promotion. In the same study, proline accumulation was enhanced by treatment with fulvic acid in both aquifers and well-hydrated plants. Peng et al. [23] reported that proline treatments with fulvic acid led to improved resistance to abiotic stress. Chen et al. [70] observed an increase in the concentration of chlorophyll in soybeans and rye grass after using fulvic acid. Zancani et al. [86] suggested that the application of fulvic acid to cell cultures of Greek fir influenced the signaling pathway of plant hormones and increased the intercellular levels of ATP and glucose-6-phosphate. Research by Azevedo and Lea [87] showed that the addition of HS affected the ability to adapt to osmotic conditions by maintaining the water absorption and cell turgor of plants exposed to drought stress.

The protection of plants against diseases caused mainly by biotic factors is another very important role of *Trichoderma* as a biostimulant and biological control agent (BCA). *Trichoderma* may act directly, controlling pathogens by antibiosis, mycoparasitism, and competition for niches and nutrients, as well as indirectly by the elicitation of defense responses and resistance in plants against pathogenic bacteria, fungi, viruses or even nematodes and insects [39, 49, 50, 71]. Depending on the strain, plant species, pathogen and soil-environmental conditions, *Trichoderma* may activate different types of resistance, that is induced systemic resistance (ISR), systemic acquired resistance (SAR) or the detected recently *Trichoderma*-induced systemic resistance (TISR), involving a wider variety of hormonal pathways interconnected in a complex network of cross-communicating signaling routes [15, 48]. TISR induction was observed in tomato plants treated with the *T. longibrachiatum* MK1 strain, protecting them against *B. cinerea* [44], as well as in melon cotyledons treated with *T. longibrachiatum,* where elicitors were able to activate both ISR and SAR pathways [45]. It is well known that the activation of defense mechanisms may use up energy and materials, thereby limiting the growth and development of plants. Therefore, *Trichoderma* strains able to simultaneously promote plant growth and induce resistance against pathogens are important potential plant biostimulants [15, 88]. Examples of a double-positive effect of *Trichoderma* were shown in different studies. For example, seeds coated with *T. atroviride* significantly improved cucumber germination, enhanced vegetative plant growth, and reduced downy mildew infection by the activation of systemic defence responses in cucumber plants [39]. Moreover, *T. harzianum* stimulated seed germination, plant growth and vigour as well as enhanced vegetative and reproductive growth parameters, including plant height, early flowering, reduced crop duration, ear head size and crop yield, and at the same time induced resistance against *Plasmopara halstedii* in sunflower plants [43]. The simultaneous growth promotion and induction of several defense-related genes, characteristic of SAR and ISR, were observed as a result of *T. longibrachiatum* influence on tomato plants, subsequently inoculated with the pathogen *B. cinerea* [44]. Additionally, a Supresivit biopreparation based on *T. harzianum* spores mixed with mineral fertilisers caused the lower infestation of spring barley, winter wheat, winter oil rape, maize, and potatoes with pathogenic fungi. Simultaneously, its positive effect on higher yields was observed [51]. In practice, *Trichoderma*-based biostimulants seem to be very important from the economical and environmental point of view [49, 50]. Applied with fungicides still used, *Trichoderma* reduce chemical doses used in integrated farming, which results in enhanced plant health comparable with the level of protection provided by the application of full fungicide doses. This fact makes it possible to reduce cultivation costs and has a positive effect on the environment [89]. Therefore, there is a need of further investigation to find the new *Trichoderma* biostimulants to be used in biopreparations in sustainable agriculture, as an alternative to chemical plant protection products.

Protein hydrolysates and specific amino acids, including proline, betaine, their derivatives and precursors can induce plant defense responses and increase plant tolerance to various abiotic stresses, such as salinity, drought, temperature and oxidising conditions [3, 56, 90-93]. Ertani et al. [29], Apone et al. [91] and Kauffman et al. [56] observed the positive effects of PH and amino acids such as proline and betaine on the secondary plant metabolism, plant defense responses and stress tolerance (salinity, drought, temperature and oxidation conditions). Ertani et al. [90] showed that the alfalfa protein hydrolysate (alfalfa PH) used for maize cultivated hydroponically under salt stress caused an increase in plant biomass, a decrease in antioxidant enzyme activity, and phenol synthesis. On the other hand, alfalfa PH may increase in proline and flavonoid contents and raise PAL activity and gene expression in relation to drought stress control. According to Colla et al. [74], the accumulation of glycine, betaine and proline is generally correlated with enhanced stress tolerance, and the exogenous use of these compounds increases tolerance to abiotic stress in many higher plants, such as corn, barley, soybean, lucerne and rice. In addition to their role in stabilising proteins and membranes, glycine, betaine and proline can scavange ROS and induce the expression of salt-responsive genes [3, 92, 94-99]. According to Lucini et al. [31], the treatment of lettuce (*Lactuca sativa* L.), which is particularly sensitive to salt, with plant-derived protein hydrolysates, increased the yield of fresh matter, dry biomass and dry root mass, as well as the concentration of osmoylites, glucosinolates and the composition of sterols and terpenes. PHs are applicable to trees that require significant investment costs and may be susceptible to drought [81]. Japanese persimmon trees, Diospyros kaki L. cv. “Rojo Brillante” grafted on *Diospyros lotus* L., are particularly sensitive to drought stress [83, 100]. Calcium protein hydrolysate treatment of plants reduced chloride uptake during saline irrigation, decreased the water potential, and also increased the concentration of compatible solutes, all of which would improve plant growth [83, 100]. Lachhab et al. [32] showed that protein hydrolysates from soy and casein can act as elicitors for strengthening vine resistance to *Plasmopara viticola*.

Biostimulant effect on fibrous plants

Despite the obvious role of biostimulants in promoting plant growth, they also affect fibre quality. The properties of fibre are related to the varieties of fibrous plants and the condition of their cultivation. Depending on the anatomical origin, there are several main types of fibres: seed fibres (cotton, kapok), bast fibres (flax, hemp, kenaf, ramie, jute, nettle), leaf fibres (agaves, pineapple, banana), fruit fibres (coir), wood (hardwood, softwood), grass and reed (bamboo, wheat, rice, oat) [101]. Fibrous plants are related to the development of ecological composites [102-104]. Natural fibres can be used for textiles, pulp and paper as a component of composites and in other industrial applications as environmental friendly materials [105-108]. Natural fibres are obtained from fibrous plants. Depending on the fibre source (plant stem, leaf, seed) and growing conditions, natural fibres can have various diameters, structures, degrees of polymerisation and crystal structures [109]. The properties of fibres are related to their chemical composition such as the presence of cellulose, hemicellulose and lignin [110-112]. Kocira et al. [113] studied the effect of different biostimulants, including seaweed and amino acids, on the content of fibre fractions in soybeans. The researchers showed that the application of a biostimulant based on seaweed and amino acids significantly influenced the level of individual fibre fractions as well as the content of hemicellulose and cellulose in the plant material.

Humic substances can display gibberellin- and cytokinin-like activities [114]. According to Silva et al. [103] cotton fibre biosynthesis may strongly depend on the overproducution of gibberellin and the plant nutritional status, under different abiotic conditions. Gibberellin can directly influence the micronaire, length and strength of the fibre [115]. Plant biostimulant treatments may increase the gibberellin content and lead to changes in fibre formation. Thus, Silva et al. [103] studied the efficiency of seed treatment with biostimulants with respect to the nutrition, yield and technological quality of cotton fibre. The application of the biostimulants increased the cotton fibre strength. Belopukhov et al. [25] studied the impact of humic-fulvic complex (HFC) on the cultivation of different fibre-type cultivars of offibre flax (*Linum usitatissimum* L.) and on the quality of the products obtained. Research showed a possitive effect of HFC application on fibre flax growth and development.

Rady et al. [24] studied HS soil application as a method to alleviate the harmful effects of salinity stress on cotton plants (*Gossypium barbadense* L.), which is a crop plant also used as a textile fibre. The researchers suggested that HS application in saline soils improved cotton plant stress-defence responses. Hanafy Ahmed et al. [116] studied the effects of putrescine and HA foliar application on the growth, yield and chemical composition of *Gossypium barbadense* L. The results indicated that in response to salt stress, cotton fibre qualities such as fibre length, fibre strength and fineness were decreased. Researchers reported that after foliar application, fibre fineness was significantly increased compared with the control sample. Bakry et al. [117] focused on the impact of humic acid and/or foliar application proline on flax plants under saline soil conditions. The results showed that HA enhanced the absorption of Fe, P and other nutritional elements, activated the defense system of the plants quickly, and increased their resistance of to environmental stresses.

In the fibre industry, enzymes released by *Trichoderma,* including commercial hemicellulases and cellulases, are used, for example, to improve the pulp properties of recycled kraft paper [118]. Lignocellulosic biomass is used as an excellent raw material for the production of fuels, chemicals and energy [119]. *Trichoderma* elicitors are also used to improve the quality of cotton and other fibrous plants [120, 121].

Among the different microorganisms positively influencing plant protection against diseases, a lot of *Trichoderma* strains were shown to induce resistance in many crops, including different fruits, vegetables and cereals. Moreover, *Trichoderma* spp. was proposed for the managment of different diseases of fibre plants, including cotton, jute, flax and coconut [42, 44, 50, 122, 123], as it protected fibre plants against the plenty of biotrophic and necrotrophic pathogens [49]. For example, some *Trichoderma* strains were strongly antagonistic towards *Alternaria,* causing leaf spots in cotton [122] and blight disease in linseed [124], or towards *Thielaviopsis paradoxa*, a fungus causing stem bleeding disease in coconut [125]. Moreover, in combination with chemical fungicides, *Trichoderma* strongly inhibited *Macrophomina phaseolina*, the causative organism of stem and root rot of jute, along with significant plant growth and fibre production promotion [126].

Conclusions

In the present review we characterised complex, multi-component biostimulants and showed their positive effect on plant growth promotion, fibre quality and on protection against different biotic and abiotic stresses. The current state of knowledge concerning biostimulant-induced resistance shows how different mechanisms may be involved in the process. Therefore, further studies at the physiological and biochemical levels are necessary to elucidate the impact of biostimulants on plants and to propose their application in order to improve crops and fibres and to protect plants against dangerous stress factors.

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