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4. Biostimulant and Biopesticide Potential in Agriculture

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Abstract:

The acceleration of climate change has devastating effects on plant growth, crop yield, as well as nutritional quality, threatening sustainable crop production worldwide. Bio stimulants and bio pesticides are agronomic tools that have been gaining importance in the reduction of fertilizer applications. Bio stimulants are known to elicit positive plant responses at lower doses than traditional fertilizers. There are various classes of bio stimulants which also included bio pesticides such as humic substances, protein hydrolysates, seaweed extracts, silicon, chitosan and microbial bio stimulants. Role of bio stimulants mainly includes nutrient acquisition, accumulation of soluble sugars and alcohol, polyamine accumulation, phytohormone regulation, amino acid accumulation, fatty acid regulation and redox homeostasis. Cellular level changes include higher photosynthetic rate, increased antioxidant activity as well as improved primary and secondary metabolism. Molecular level changes are associated with increase in gene expression of antioxidants, nutrient transporters and stress related genes. Phenotypical modification includes improved root and shoot growth, higher yield parameters, better nutritional quality and increase in vigor. There is a rising demand for a solid scientific foundation to lay the groundwork for a theoretical framework that would help in the development of bio stimulant products with credible scientific descriptions. In recent years, omics approaches such as genomics, transcriptomic, proteomics and metabolomics have proven to be instrumental in the investigation and identification key multi-layered *biochemical events and mechanisms as effected by bio stimulant formulations on physiology of plants. Using this combined approach, it is possible to develop highly efficient bio stimulants and bio pesticides contributing to better agricultural practices.*

Keywords:

Humic substance, protein hydrolysates, seaweed, mode of action, omics, phenotyping.

4.1 Introduction:

Bio stimulants are agronomic tools that have been gaining importance in the reduction of fertilizer applications. The beneficial effects of plant bio stimulants are usually not due to their nutrient content but to regulatory effects on the plant's metabolism. Bio stimulants are known to elicit positive plant responses at lower doses than traditional fertilizers.

One the major classes of bio stimulants are bio pesticides, which are derived from animals, plants and microorganisms like bacteria, cyanobacteria, and microalgae. They are applied to agricultural pests and diseases.

Bio pesticides can significantly enhance the use and application of agro-based chemicals in the agricultural sector to protect crop plants from invasive and infecting pests. A major advancement towards ensuring sustainable food production for future, is the application of bio stimulants and bio pesticides, which have been seen as a novel approach by stimulating plant growth and productivity under both stress and normal conditions (Rai *et al*., 2021).

The composition of these compounds is primarily responsible for their effectiveness, and determining their efficacy continues to be a crucial aspect that complicates the characterization process.

Therefore, elucidation of the biological basis of bio stimulant functions and the development of scientifically based bio stimulants lead to effective exploration and application of formulations in agriculture.

4.2 Bio Stimulants and Its Classification:

Ministry of Agriculture and Farmer Welfare, Department of Agriculture, Co-operation and Farmers Welfare issued the Fertilizer (Inorganic, Organic, or mixed) (Control) Amendment Order (FCO), 2021, which sets down the regulatory framework for bio-stimulant registration in India.

As per the order, a 'bio-stimulant' denotes a substance or micro-organisms or a combination of both whose function when applied to plants, seeds or rhizosphere is to stimulate various physiological processes in plants and further enhance its nutrient uptake and efficiency, growth, yield, crop quality and tolerance to stress, regardless of its nutrient content, but does not include plant growth regulators or pesticides which are regulated under the Insecticides Act, 1968.

4.2.1 Humic Substances:

Humic substances (HS) are the primary source of organic carbon, created by biological and chemical metabolism of microbial processes involving plant and animal debris. HS sustain plant growth and terrestrial life in general, regulate both soil carbon and nitrogen cycling, the growth of plants and microorganisms, the transport of anthropogenic-derived compounds and heavy metals and the stabilization of soil structure. It is more accurate to describe HS as a group of varied, generally low molecular mass constituents that dynamically establish associations and are stabilized by hydrogen bonds and hydrophobic interactions.

4.2.2 Protein Hydrolysates (PHs):

Protein hydrolysates are mainly produced by chemical (with strong acids or alkalis) and/or enzymatic hydrolysis of proteins contained in agro-industrial by-products from animal (i.e., leather, viscera, feathers, blood) or plant origin (i.e., vegetable by-products) and in biomass of dedicated legume crops (i.e., seeds, hay). Applying PHs to leaves and roots has been demonstrated to improve micronutrient mobility and solubility, specifically, Zn, Fe Mn, and Cu through chelation and modifications in root architecture, as well as water and nutrient uptake and nutrient use efficiencies for both micro and macro elements (Halpern *et al*., 2015) This is because of the increase in enzymatic and microbial activity in soil, and increase in nitrate reductase, glutamine synthase, and Fe(III)-chelate reductase activities(Lucini*et al*., 2015).

4.2.3 Seaweed Extracts:

Seaweed extracts have now gained much wider acceptance as “plant bio stimulants”. Seaweed extracts, generally induce a wide range of responses in plants, even at low concentrations. These responses include enhanced plant growth, improved flowering and yield, improved product quality, improved nutritional content in edible products, and extended shelf life. Seaweed extracts alter physical, biochemical and biological properties of the soil and affect root architecture facilitating efficient nutrient uptake.

Brown seaweeds contain polyuronides such as alginates and fucoidans. Alginic acid showed soil-conditioning properties and also chelated metal ions forming polymers of high molecular weight (Battacharyya*et al*., 2015).

4.2.4 Silicon:

Silicon is a ‘semi-essential’ element for higher plants, in the sense that plant growth may be stimulated by enhanced Si supply of, while Si-starved plants may exhibit physical abnormalities. Silica deposition in cell walls causes anatomical changes in plant tissues, which may be the reason of some of Si's advantageous effects. Due to the deposition of photoliths, which give plants rigidity and regulate water and nutrient mobility, silicon causes anatomical changes in plant tissues. It also strengthens antioxidant defense systems, immobilizes toxic metals through complexation or co-precipitation with Si in plant tissues and soil, and modifies gene expression and signaling through phytohormones.

4.2.5 Chitosan:

Chitosan is the deacetylate form of chitin, that naturally occurs as a biopolymer in fungal cell walls, insect exoskeletons and crustacean shells. Research has examined the physiological and biochemical reactions of chitosan and discovered that it functions as a stimulant of defense responses in plants. In order to promote plant growth, abiotic stress tolerance, and pathogen resistance, chitosan can also be used as a bio stimulant. Nevertheless, these reactions are complex and rely on various chitosan-based structures and concentrations, in addition to the type of plant and stage of development.

4.2.6 Microbial Bio Stimulants:

Microbial bio stimulants are composed of microorganisms such as plant growth-promoting rhizobacteria (PGPR) and/or microbes which stimulate nutrient uptake, produce secondary metabolites, siderophores, hormones and organic acids, participate in nitrogen fixation, imparts stress tolerance, enhance crop quality and yield when applied to the plants.

AMF symbiosis not only to improve plant nutrition by interacting with bi and trivalent cations but also interfere with the phytohormone balance of the plant, thereby influencing plant development and alleviating the effects of environmental stresses (Kaushal *et al*., 2023). Plant Growth-Promoting Rhizobacteria, or PGPRs, are multipurpose microorganisms that affect every facet of plant life, including interactions with other organisms in agro-ecosystems, nutrition and growth, development and morphogenesis, and response to all kind of stress. They play a role in the bio-geochemical cycles, nutrient supply, improved nutrient utilization, disease resistance induction, increased tolerance to abiotic stress, and morphogenesis modulation by plant growth regulators.

Table 4.1: Commercially Used Bio Stimulants and Bio Pesticides and Its Implications

Sr. No.	Bio Stimulants	Beneficial Effect
1.	Humic and Fulvic acid	Increases soil health by boosting macronutrients, micronutrients, and beneficial microorganisms, all of which improve soil fertility. It functions as organic manure, increasing the soil's ability to hold water. promotes the development and procreation of advantageous soil microorganisms
2.	Protein hydrolysates	Improvements in the nitrogen uptake and assimilation an increase in the soil's overall fertility, and an increment in the biomass and of soil microbial activity.
3.	Seaweed extracts	Rise in the overall fertility of the soil, modifications to the assimilation and uptake of nitrogen, and an increase in the biomass and activity of the soil microbial population
4.	Chitosan	Generation of active oxygen species, cellular responses like ion flux variations and membrane depolarization, cytosol acidification and changes in protein phosphorylation

Sr. No.	Bio Stimulants	Beneficial Effect
6.	Megafol- by Syngenta (Amino acid-based bio stimulant)	Encourages balanced vegetative growth and productivity; by overcoming stressful conditions, it maintains the yields' quality and quantity.
7.	Ambition- by Bayer (Amino acid and fulvic acid)	Stimulate plant growth on new shoot, flowering, fruit formation and harvest. Quickly recover plants from biotic/abiotic-induced stress and better nutrient absorption.
8.	Biplantol universal by BNG (Macro-and microelements, germanium, uronicacids, medicinal herb)	Increases the strength and vigor of plants by promoting the soil's microbiota, removing obstructions within the plant, and starting the sap flow. The plant can better absorb nutrients and deliver them to each and every cell by growing more roots. As a result, the plant becomes more robust, healthy, and resistant to disease and pest infestation.
9.	SPICem Power-G - by SPIC (Humic acid, amino acid and fulvic acid)	Prolongs fruit shelf life by promoting photosynthesis and aiding in the thickening of the fruit's epidermis. It enhances the plant's physiological, chemical, and biological processes to yield high-quality, high-yield produce. enhances the produce's aesthetic appeal and nutritional content.
Sr. No.	Bio Stimulants	Beneficial Effect

(Madende and Hayes, 2020)

4.3 Role of Bio Stimulants and Bio Pesticides in Regulating Growth:

4.3.1 Accumulation of Soluble Sugars and Sugar Alcohols:

It is commonly known that sugars play an important role as osmo protectants in tolerance to various stresses, such as trehalose, sucrose, and fructans. Sugars are the main osmolytes that many plant species use to change the osmotic pressure. Sugars like fructans, trehalose, and raffinose preserve membrane integrity and stabilize proteins. In order to strengthen the abiotic stress tolerance mechanism, bio stimulants promote the accumulation of these soluble sugars and alcohols.

4.3.2 Endogenous Phytohormone Regulation:

Phytohormones are signalling molecules, produced in low quantities, control local and distant developmental processes in plants. This bio stimulant works by upregulating (inducing) and downregulating (inhibiting) hormonal activity. And can be used to promote plant development at different crop stages Stress prevents the synthesis of gibberellic acid

(GA), restricting the plant's energy required for vertical development. But the synthesizing of auxins, brassino steroids (BR) and jasmonic acid (JA) increased. It is well known that BR signalling, which typically boosts the biosynthesis of ABA and auxin, inhibits the production of JA.

Both ABA and auxins have a role in regulating stomatal aperture. Cytokinin (CK) synthesis is suppressed by SA and ABA signalling (Salvi *et al.*, 2021). The synthesis of ethylene, cytokinin (which blocks auxins in root development) and abscises acid -related substances could be down regulated by the application of bio stimulants.

4.3.3 Polyamine and Amino Acids Accumulation:

Because of their capacity to stabilize cell membranes, scavenge free radicals, and neutralize acids, low-molecular-mass polycations known as polyamines are widely found in plants and have a demonstrated ability to prevent ageing and stress. A wide range of amino acids can be used as building blocks to produce signalling molecules and secondary metabolites.

Whereas, research studies have highlighted the significant roles of proline, GABA (gamma-aminobutyric acid), glycine betaine, serine, methionine, as well as polyamines and aromatic amino acids (Trovato*et al.*, 2021) under the influence of bio stimulants application.

4.3.4 Fatty Acid Regulation:

Hydrocarbon chains with a carboxyl group at one end are called fatty acids. Sterols, fatty acids, and sphingolipids are examples of lipids that have negative effects on biosynthesis. The use of bio stimulant treatments seems to lessen or even reverse these effects.

Lipid-dependent signalling cascades are known to be initiated by linoleic acid and its derivatives, such as hexadecadienoic acid (HDDA) and hydroperoxyoctadecadienoic acid (HPDA). These cascades initiate responses in plants to adapt by mediating the expression of genes linked to defense mechanisms.

4.3.5 Glutathione and Ascorbate Synthesis:

Two interrelated defense systems have been evolved in plant against oxidative stress: enzymatic and no enzymatic detoxification. The main detoxification mechanism used by different cellular organelles to counteract an excessive build-up of harmful oxygen radicals is the glutathione ascorbate pathway. Ascorbate contributes to the maintenance of metal ions' reduced state, which is necessary for antioxidant enzymes to function as cofactors and maintain their activity. (Hasanuzzaman *et al.*, 2019). Glutathione’s biological properties as an antioxidant are primarily attributed to cysteine, which plays a role in preserving cellular redox balance.

Bio stimulant application has been shown to improve osmoregulation, the water and nutrient status of plants, their ability to photosynthesize, their efficiency in using water, and, generally, their overall productivity by controlling the antioxidant defense machinery.

4.4 Mechanism of Action of Bio Stimulants:

Bio stimulants play an important role in crop production by altering the plant's responses through various physiological and biological processes including ROS scavenging, some protection, membrane stability, stomatal guiding, xylem conductance, metal chelation, nutrient and water availability and phytohormonal signaling in plants (Oosten *et al*., 2017). The macroscopic algae, *Ascophyllum nodosum* is utilized in many different products because of its high polysaccharide and phenolic content. Bioactive components extracted from *A. nodosum* were discovered to control multiple post-transcriptional and post-translational regulation steps of distinct TFs, including MYB, LHY1, AP2/ERF, WRKY, NF-YA, COR15A (Goni*et al*., 2016). The application of proline and betaine to *Hordeum vulgare* reduces the NaCl-induced efflux of potassium ions. By promoting the potential for acid growth, hormonal regulation, and nutrient uptake, it alters H+ ATPases on the plasma membranes, which has resulted in more than 40% of proton extrusion (Zandonadi *et al*., 2016). Along with an increase in phenylpropanoids, terpenes, flavonoids, nitrogen-containing compounds, gluconates, and alkaloids, it also results in increased concentrations of auxin, gibberellins, and cytokinin. Humic acid substance has also been linked to hormone-like behaviour because it contains auxin. Under HA treatment, there can be changes in gene expression of PM-H+-ATPase (Mh1), which is essential for the electrochemical gradient of cell membranes that leads to improved nutrient absorption, aquaporin 1 (PIP1), which helps with the molecular transport of water and solutes, and nitrate transporters (Nrt2.1 and Nrt1.1). Fulvic acids appear to up-regulate genes linked to lipid biosynthesis, K transporters, starch degradation, and plant metabolism, which results in an increase in lipid content. (Pylak*et al*., 2019). Microbial bio stimulants have been found to enhance the growth of several crops and vegetables, including lettuce, peppers, beans, and tomatoes (Mayak*et al.,* 2004). They cause the host plants to grow longer roots, which improves water and mineral uptake. Some PGPR possess the enzyme of 1-aminocyclopropane-1-carboxylate (ACC) deaminase that can scavenge plant ACC (Orozco-Mosqueda et al., 2020). These bacteria hydrolyze ACC to ammonia and α-ketobutyrate, thereby monitoring excess ethylene concentration.

4.4.1 Three Stages of Bio Stimulants Action:

A. Penetration into Tissues, Translocation and Transformation in Plants:

Bio stimulants applied to the plants has to be penetrated into the tissues, translocated through the plant sap and induce appropriate signalling mechanism. Foliar application of bio stimulants is mainly taken up through stomata and in case of soil application, they are taken along with water/nutrients or mediated through transporter proteins.

B. Gene Expression, Plant Signaling and The Regulation of Hormonal Status:

Bio stimulants have been shown to induce genes and benefit productivity only when plants are challenged by abiotic and biotic stress. The bioactive compounds in some bio stimulants are proposed to display signaling activity in plants or induce signaling pathways. Amino acids, glycosides, polysaccharides and organic acids contained in many bio stimulants act as precursors or activators of endogenous plant hormones.

C. Metabolic Processes and Integrated Whole Plant Effects:

Bio stimulants improve plant productivity through increased assimilation of N, C and S, improved photosynthesis, improved stress responses, altered senescence and enhanced ion transport.

4.4.2 Knowledge Gap Pertaining to Bio Stimulants:

Agronomic and phenotypic traits have typically been the focus of the majority of bio stimulant research; however, the cellular and molecular mechanisms underlying these agronomic allies observed benefits are poorly understood. A product's degree of efficacy and effectiveness must be credibly described if it is to be recognised as a bio stimulant. As a result, there is a growing need to identify the scientific description of the mechanism of action of bio stimulants.

4.5 Modern Approaches to Determine the Role of Bio Stimulants:

The most recent European fertilizer regulation and the recommendations of the European Bio Stimulants Industry Council (EBIC) state that registering a product as a plant bio stimulant requires providing scientific proof of the bio stimulants' effectiveness as well as elucidating their unique mechanism of action. Trails must be conducted both under protected condition and in the open field, on model plants such as *Arabidopsis thaliana* or tomato cultivar Micro-tom and other non-model species.

Different techniques, including in vitro bioassays, micro-phenotyping, high-throughput phenotyping and omics studies have been established and replaced traditional time-consuming screening methods.

For determining the mode of action of these plant bio stimulants, screening platforms based on automated or semi-automated bio assaying of plant/tissue characteristics using straightforward read-outs may be helpful. The multi-dimensional phenotype data obtained from the integrative phenotyping approach can be statistically analysed and advanced data analysis algorithms can be used to cluster the morpho-physiological traits and identify the traits that correlate with the stress response or the specific phase of bio stimulant application. Regarding bio stimulants, the variables of interest that are currently observable through field phenotyping systems associated with photosynthesis, water relations, canopy structure and growth, and leaf biochemistry.

- Ground based lidar or terrestrial 3D laser scanning system- canopy structure assessment
- Infrared thermography- transpiration and stomatal functional
- Fluorescence imaging- photosynthetic functioning

Transcriptome analyses quantify the changes in gene expression brought about by the bio stimulant-plant interaction. A transcriptomic fingerprint is produced by microarray analysis, which enables the simultaneous study of several genes. The resulting profiling is used to identify the target gene families as well as to classify and cluster the genes into functional groups based on the levels of their expression. Understanding the function of humic acids in preventing various unfavorable situations before they arise was made possible by RNA-sequencing. Tomato plants expereicnedless salt stress after application of bio stimulant with tannins called VIVEMA TWIN by controlling the expression of crucial transcription factors and genes that respond to stress (Campo Benedetto *et al*., 2021).

Proteomics analysis makes it possible to spot changes in the protein profile and locate post-translational modifications, which are vital to figuring out the biochemical and molecular processes involved. In this view, a proteomic study conducted on tea plants exposed to drought conditions revealed a positive effect of fulvic acid (Qiu*et al*., 2021).

Finally, a correlation with a transcriptomic analysis permitted to highlight that the expression patterns of 55 genes were similar to those observed at protein level. Metabolomics, a comprehensive qualitative and quantitative study involving, metabolites that are the ultimate recipients in the flow of biological processes.

A seaweed extract-based bio stimulant (*Dunaliella salina* exopolysaccharides) was applied to tomato plants to investigate its role in the amelioration of saline stress. By using metabolomics, it was determined that *D. salina* contains bioactive substances called sulfated exopolysaccharides. These substances were shown to alleviate salt stress by upregulating proline, phenolic, osmoprotectant compounds, and antioxidant enzyme activity.

4.6 Bio Stimulants Market:

According to current trends, the global bio stimulant market is expected to grow from its 2022 valuation of USD 2.6 billion to USD 8.5 billion by 2027, with a Compound Annual Growth Rate (CAGR) of 11.8%.

The India Bio Stimulants Market is segmented by Form (Amino Acids, Humic Acid, Fulvic Acid, Protein Hydrolysates, Seaweed Extracts) and by Crop Type (Cash Crops, Horticultural Crops, Row Crops) with CAGR of 10.42%. The bio stimulants based amino acids are the fastest-growing form.

It is estimated that in 2023-2029, the amino acid substances occupy 13.53 % in the market, followed by fulvic acid, humic acid, protein hydrolysates and seaweed extracts (largest growing form).

As per the Fortune Business Insights report, the majority of bio stimulant products are applied to crops as foliar treatments, which is the most common method of application. Soil and seed treatments are the next most common methods (Fortune business insights, 2023).

The top five companies in the India bio stimulants market hold 10.77% of the market, which is fragmented.

Biostadt India Limited, Coromandel International Ltd., Southern Petrochemical Industries Corporation, T. Stanes and Company Limited, and Val agro are the leading companies in this market.

4.7 Conclusion:

Under climate change scenarios, securing and optimizing global agricultural production is crucial. To help achieve this goal, agronomic techniques, conventional and modern breeding techniques, and genetic engineering have been developed. In this scenario, high agricultural productivity has been attained worldwide through the use of bio stimulants, which also lessen the negative effects of agrochemicals and climate change. Even though bio stimulants are widely acknowledged to be effective and have bright futures, there are still a lot of issues that need to be resolved, including the complexity of their complex composition, the difficulty of defining and commercializing their products, and the lack of effective production technology for high-quality products.

Combined approach using biology, chemistry and omics might pave way for developing highly advantageous bio stimulants contributing to better agricultural practices.

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