Biostimulant: an innovative approach for sustainable crop production

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Biostimulant is a substance or microorganism, or a combination of both, which stimulates the physiological activities of plants, leading to increased uptake and efficiency of nutrients, crop quality and tolerance to stress related to biotic and abiotic factors. Biostimulant helps optimise crop productivity and enhances the quality of the produce, which help maintain an eco-friendly environment to sustain agricultural production. Various botanical extracts, biochemicals, protein hydrolysates and amino acids, vitamins, cell-tree microbial products, antioxidants, anti-transpirants, humic and fulvic acids, and their derivatives are included under biostimulants. Recently, the fertilizer (inorganic, organic or mixed) Control Amendment Order, 2021, has been passed by the Government of India to promote biostimulants in the country. The Central Biostimulant Committee advises on various matters related to biostimulants. Participation of stakeholders, farmers, researchers, policymakers and regulators is essential to popularize biostimulants for their profitable and sustainable usage. This article focuses on various aspects of biostimulants, including regulatory aspects in India.

Keywords: Biostimulants, innovative approach, metabolic enhancers, regulatory aspects, sustainable crop production.

BIOSTIMULANT is a substance or microorganism, or a combination of both which stimulates physiological activities and enhances nutrient uptake and efficiency, growth, yield, crop quality and tolerance to stress in plants. It also reduces the requirement for fertilizers. Application of biostimulants in small amounts enhances the physiological processes in plants, leading to high and quality produce[1]. The term 'metabolic enhancers' has been used for biostimulants[2]. They enhance the metabolic and enzymatic processes of plants, especially in the early stage of development. They also increase nutrient uptake, making the plants more tolerant to stress and enhancing yield. Therefore, these substances play an important role in the structural processes in plants in relation to improving their growth, tolerance to abiotic and biotic stress and increasing yield and quality of the produce[3,4]. Biostimulants affect physiological activities and provide better growth and development in plants in response to toxic elements, water and saline stress[5].

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The first global congress to discuss the importance and use of biostimulants of agriculture was conducted in Strasbourg, France, in November 2012. This was a landmark event to attract the attention of various stakeholders on biostimulants in agriculture. In addition, biostimulants are becoming a component of production systems to modify physiological processes in plants for optimum productivity. They are receiving substantial attention from both the scientific fraternity and commercial enterprises[4,6]. Biostimulants offer an innovative approach to regulating and modifying physiological activities in plants to increase growth, tolerance and yield. Nowadays, biostimulants and biological-origin substances are gaining importance in agriculture due to the requirement for quality produce, better yield and an eco-friendly environment for sustainable production. This article aims to provide comprehensive information on biostimulants, their uses, and the scientific framework to be adopted for further work to make them popular among the farming community.

Categories of biostimulants

Plant biostimulants are primarily divided into two major categories, namely substance and microorganism, by various stakeholders, regulators and scientists[5,7,8]. They can also be classified based on the origin of active substances, mode of action[9] and their physiological responses in plants[10]. Biostimulants have also been classified on the basis of their sources and ingredients, such as humic substances, hormones and amino acid-containing products[3]. They are generally diverse in origin and described under eight categories, namely humic substances, complex materials of organic origin (waste compost, extract of sewage sludge and manures), beneficial elements (Co, Se, Al, Si and Na), inorganic salts such as phosphite, seaweed extracts (macroalgae), chitin and chitosan derivates, anti-transpirants (polyacrylamide and kaolin), free amino acids and N-containing substances[5]. Similarly, six non-microbial biostimulants, viz. chitosan, humic and fulvic acids, hydrolysates protein, seaweed extracts, silicon and phosphites, and three microbials, viz. arbuscular mycorrhizal fungi (AMF), *Trichoderma* species and plant growth-promoting rhizobacteria (PGPR) have been proposed as biostimulants[11]. They may also

be classified on the basis of their functions and claim in agriculture, including several natural substances like fulvic and humic acids, vegetative and animal proteins, hydrolysates, seaweeds, microalgae, silicon, beneficial microorganisms like AMF, and N-fixing bacteria such as *Azotobacter*, *Rhizobium* and *Azospirillum*[12].

In 2021, the Government of India (GoI) issued a Gazette notification (Gazette of India: Part II-Sec. 3-ii), in which the biostimulants specified in schedule VI were grouped into various categories, namely biochemicals, vitamins, protein hydrolysates, antioxidants, anti-transpirants, humic and fulvic acids and their derivatives, botanicals, including seaweed extracts and cell-free microbial products[13]. However, several raw ingredients are being used as bioactive compounds in biostimulants, namely acids (fulvic and humic), algae and seaweed extracts, vitamins, amino acids (arginine, alanine, glycine, proline, hydroxyproline, serine, glutamic acid, phenylalanine, aspartic acid, valine and tryptophan), ascorbic acid, plant hormones (gibberellic, auxin, cytokinins, kinetin), calcium, salt (NaCl) concentration, protein hydrolysates, AMF and PGPR.

Humic and fulvic substances

Humic substances are heterogeneous compounds which categorized according to their molecular weights and solubility into humins, humic acids and fulvic acids. They are organic due to the decomposition of plant, animal and microbial residues. Therefore, their ingredients are natural in origin and essentially require to improve soil fertility, help in ameliorating root nutrition and enhance cation exchange capacity of the soil. They help in nutrient uptake and increase availability of phosphorus due to calcium phosphate precipitation. Humic acid extracts play an important role in stress tolerance for salinity, increase endogenous proline levels and reduce membrane leakage[14,15]. They also enhance the function of antioxidants and reactive oxygen species scavenging enzymes, thus inactivating the production of toxic-free oxygen radicals in plants under abiotic stress[16,17].

Protein hydrolysates and N-containing compounds

Protein hydrolysates and N-containing compound mixtures enhance basic nutrition and growth[1]. Amino acid and peptide mixture from plant and animal sources enhance growth in plants[5,7,8]. Glycine betaine has anti-stress properties and maintains osmotic potential in plants[18]. The accumulation of glycine betaine makes plants tolerant to abiotic stress. Enzymatic hydrolysis of porcine haemoglobin reduces the influence of thermal and cold stress in plants. Porcine haemoglobin reduces the negative impact on plant growth due to increasing temperature[19].

Seaweed and algal extracts

The use of seaweed and algal extracts as organic matter and fertilizers is well-known in agriculture. These include polysaccharides (laminarin and alginates) and carrageenan's micronutrients and macronutrients, sterols and N-containing compounds[6,20]. The algal species belong to the genus *Ascophyllum*, *Fucus* and *Laminaria*. They are applied in the soil as a hydroponic solution and foliar spray[6,20,21]. Seaweed extracts improve the tolerance of plants under extremely low and high temperatures[22]. Algae extracts improve basic nutrition, plant growth and development, and contribute to water retention, fixation and exchange of cations and soil remediation[1]. The application of algae extract significantly increased the total chlorophyll contents and antioxidants in plants. These parameters showed a positive correlation with increased grain weight and yield[23–25].

Chitosan and other biopolymers

Chitosan is produced by the deacetylation of the polymer chitin. Chitosan application helps develop tolerance against fungal pathogens, abiotic stress and enhances quality traits related to primary and secondary metabolism. Chitosan oligomers has the capacity to bind with a wide range of cellular components, including DNA, plasma membrane and cell wall constituents and to bind specific receptors involved in defense gene activation[26–29]. Stomatal closure induced by chitosan provided protection from the environmental stress[30]. Accumulation of hydrogen peroxide and Ca^{2+} leakage into the cell contribute significantly to physiological changes, which play an important role in the signalling of stress responses and regulation due to the cellular binding of chitosan to receptors. Studies on the transcriptome and proteome of plant tissues treated with chitosan have confirmed the same[31,32].

Inorganic compounds

Many beneficial elements are reported to enhance growth, quality of produce, and tolerance to abiotic stress in plants. Such beneficial effects include cell-wall rigidification, osmoregulation, transpiration reduction by crystal deposits, thermal regulation by radiation reflection, activities of enzymes, antioxidant protection, plant nutrition, protection against heavy metals toxicity, hormones synthesis and signalling, interactions with symbionts, pathogens, and herbivore response[33]. Inorganic elements, namely chlorides, silicates, phosphates, phosphites and carbonates, are used as fungicides[34]. Silica deposition during pathogen attack and osmotic stress in grass are enhanced by applying inorganic elements and amorphous silica ($SiO_2 \cdot nH_2O$). This might be due to the influence of hormone signalling enzymes involved in stress response and redox homeostasis, pH, osmotic pressure, nutrient efficiency, plant growth and tolerance to

abiotic stress. It has been reported that NaCl salt concentration increases seed germination of parsley, leek, tomato, onion, celery, lettuce, basil and radish[35].

Beneficial fungi

Beneficial fungi play an important role in enhancing tolerance against biotic and abiotic stress in plants while interacting with their roots and establishing a symbiotic relationship. The association with plant roots may be parasitic also[36]. Both plants and fungi had co-evolved during their terrestrial evolution. The concept of mutualism–parasitism continuum is useful to describe the extended range of relationships that developed over the time of evolution[37]. Mycorrhizal fungi benefit plant growth and development, and more than 90% of plant species have a symbiotic relationship. AMF, commonly known as endo-mycorrhiza, are widely distributed and associated with various crop plants. The hyphae of AMF produce arbuscules, a special structure penetrating the cortical cells of roots and establishing a symbiotic relationship with the plants to enhance nutrient and water use efficiency, tolerance to biotic and abiotic stress and crop yield and quality. Several other fungal species, such as *Trichoderma* and *Sebacinales,* have also established a strong endophytic relationship with plants, enhancing nutrient uptake to their host and growing tolerance to biotic and abiotic stresses[36]. The application of AMF, other beneficial fungi and algae as raw materials of bioactive constituents improved tolerance against salinity in plants due to enhanced seed germination, growth of roots and shoots, productivity and quality of yield[38,39].

Beneficial bacteria

Beneficial bacterial species play an important role as biostimulants. They are associated with the plants as endosymbionts like *Rhizobium* species or PGPRs like *Pseudomonas* species. *Rhizobium* species are being used as biofertilizers. PGPRs influence nutrition and growth, morphogenesis and development, response to biotic and abiotic stress and interactions with other organisms in the agroecosystems[40–43]. *Pseudomonas putida* strain AKMP7 proved to be thermotolerant and significantly increased shoot and root length, plant biomass, heat tolerance and seed size in wheat[44].

Plant hormones

Plant hormones, like auxin, gibberellic acid, cytokinin and kinetin in low concentrations, enhanced nutrient efficiency, traits related to crop quality and tolerance to abiotic stress[1,45,46]. Auxin is an important hormone that regulates plant growth and development by inducing cell division and differentiation, apical dominance, senescence, abscission and flowering. Gibberellic acid has a significant influence on the process of seed germination, mobilization of endosperm, activities of enzymes such as protease, alpha-amylase, dormancy breaking, elongation of the stem, division of cells and growth. Seedling emergence, growth and size of the leaves were found to increase by applying cytokinin and indole-butyric acid. Application of kinetin and calcium sustained relative water content and reduced cellular electrolytes[45].

Regulatory steps in India for biostimulant commercialization

In India, the use of biostimulants is regulated by the Fertilizer (inorganic, organic or mixed) Control Amendment Order, 2021. Pesticides and plant growth regulators listed under the Insecticide Act, 1968 (46 of 1968) are not included as biostimulants. An application for provisional registration of biostimulants should be submitted in Form G-1 along with the required documents. The Controller will provide a provisional certificate of registration in Form G-3. The manufacture or import of biostimulants is included in Schedule VI. Form G describes application format for including biostimulants in Schedule IV. Prior to manufacture or import, the inclusion in Schedule VI is essential. Every container in which any bistimulants is packed should have the word ‘Biostimulants’.

In India, each manufacturer or importer involved in biostimulant commercialization needs to submit an application to the Controller with data relating to the product as a biostimulant with specific information as mentioned in the Gazette of India: Extraordinary CG-DL-E-24022021-225410 Part II-Sec. 3-ii dated 23 February 2021 (ref. 13). These include biostimulant chemistry such as its source (natural extracts of the plant, microbes, animal and synthetic, etc.), specification of the product with analysis obtained from the National Accreditation Board Laboratory (NABL) or Good Laboratory Practice (GLP) for testing and calibration, properties of active ingredients (physical and chemical) and adjuvants, and method of analysis to confirm the specifications and shelf-life. Bio-efficacy trials should be conducted by the National Agricultural Research System (NARS), which includes the Indian Council of Agricultural Research (ICAR) or State Agricultural Universities (SAUs) with a minimum of three dosages of biostimulants to be evaluated at three different agro-ecological locations for one crop season. The data on toxicity is also required along with the application. Toxicity tests recommendations namely, acute oral, dermal, inhalation (rat), skin and eye irritation (rabbit) along with toxicity to birds, fish (freshwater), honeybee and earthworm are required. The manufacturer or importer should submit a product sample, the report of heavy metal analysis and an affidavit on non-judicial stamp paper with a declaration that the product is not laced with pesticides beyond the permissible limit of 0.01 ppm. The maximum limit of heavy materials is 5–1000 mg/kg. The

essential data requirements for biostimulants having a natural origin or others shall be decided by the Central Biostimulant Committee (CBC). The Committee advices GoI on various matters related to biostimulants, namely a new biostimulant inclusion, sample drawing and analysis methods, biostimulant specifications, laboratory requirements, evaluation of biostimulants and any other matter which is referred to CBC by GoI.

Legal framework in the European Union

The regulatory status of biostimulants in Europe is complex. This is because of the non-existence of a harmonized and specific framework. The European Commission regulation on plant production products (PPPs) is applied to biostimulants. They are being promoted and marketed by national regulation on fertilizers and European pesticide law[4]. Both international and national provisions, in combination, introduce PPPs in the market[4]. Biostimulants are being used on many crops covering most cereals, pulses, oilseeds, vegetables and fruits[47].

Challenges and the way forward

The major challenges to biostimulants are regulatory, scientific and technical aspects[4]. The complex nature of the physiological effects of biostimulants is the main challenge for scientific work. Biostimulants induce physiological responses like primary metabolism, growth and development in plants. The development of physiological responses is subjected to limited homeostatic regulations related to biological evolution, which took millions of years. The evolutionary process determines the specific ecological niches of plants and display characteristic phenotypic responses to fluctuating environments. The successful use of biostimulants should be based on a thorough understanding of the tripartite interaction among biostimulants, plants and the environment. Proper attention should be given to understanding the crosstalks happening between processes and pathways in plants and organisms in response to the environment. The formulation and blending of biostimulants with other chemical substances are the major challenges related to technical aspects. The interactions between microbial components of the biostimulant mixture and between the biostimulant and resident rhizospheric/endospheric microbiota are complex and need further attention. Biostimulants provide benefits, both immediate and delayed, to the farmers, including saving resources and ecosystem services. Also, it is difficult to distinguish between biocontrol agents and biostimulants because of their similarities and common modes of action.

Although India has a regulatory framework for biostimulants, there are several challenges related to the classification of biostimulants, premarket assessment and intellectual property rights (IPRs). Some of the biostimulants overlap with other regulations. Therefore, for such categories, specific regulations are not mentioned in national and international laws. The regulations related to fertilizers and pesticides promote the marketing of biostimulants and make them a distinct regulatory category. Appropriate directives and regulatory certainty in the efficacy and risk assessment of biostimulants are important for the development of technology and marketing of the products. The linkage between biostimulants and fertilizers has helped in the marketing of products across the world. Market harmonization at the national and international levels is another major concern. Patentability and prevention of copies/reverse engineering of the biostimulant products are often difficult. Proving the novelty of the product invention process is often difficult during patent processing for a biostimulant. Compulsory registration of biostimulants, along with data protection, are required to strengthen the IPR issues. Simultaneously, a mechanism should be established for sharing data during the registration of biostimulants for market support to promote exchange and partnership among the industries.

Pros and cons of biostimulants

Globally, abiotic stress causes heavy crop loss. Biostimulants are being integrated into the production system to modify physiological processes in plants to a great extent to achieve higher production while preventing loss due to abiotic stress. Biostimulants help provide favourable plant growth conditions and regulate water and nutrient efficiency to improve plant growth. Thus, they are considered a promoter of productivity by modifying physiological processes in plants. In addition to providing basic nutrition to the plants, most biostimulants like algal extracts, protein hydrolysates, humic and fulvic acids, etc. help plants tolerate abiotic stress and often enhance plant growth. Application of biostimulants as a seed treatment or during early plant development stages stimulates root development and growth, especially under low water and nutrient availability in the soil, and also enhances seed germination and seedling vigour. Several biostimulants are derived from recycled waste products which are not based on scientific principles and efficacy. Several product evaluation studies indicated their ineffectiveness, unstable and inconsistent properties[48–52]. Foliar and root application of a product containing amino acid of animal origin showed an inhibitory effect on plant growth and iron nutrition[51]. Similarly, several biostimulants were evaluated against plant pathogens, and none proved effective in achieving a sufficient degree of control. Thus, they need supplementation with conventional fungicides to achieve the desired level of protection against pathogens[50].

Conclusion and future perspectives

The development and use of biostimulants have great potential under the present agricultural scenario. They modify

physiological processes in the plants so as to enhance growth and increase yield. To meet the increasing demand of quality products, eco-friendly environment and sustainable nutrient and water management, biostimulants should be popularized in agriculture. Recently, more emphasis has been given to the development and use of biostimulants, which is evident from the number of publications, scientific conferences and symposia, and enactment of legislation. However, the industries are facing two major problems with regard to biostimulants. The first is related to difficulties in identifying the primary mode of action due to highly complex and incompletely identified compositions of biostimulants. Second, is the classification for regulation of biostimulants, which is based primarily on the source instead of the biological mode of action. Effective monitoring procedures are required for the efficacy assessment of biostimulants. Also, biostimulants derived from by-products must be promoted instead of chemical fertilizers and pesticides. The mode of action needs to be studied in detail. Basic research is needed to decipher the transcriptomic and proteomic humic substances to understand the mechanism of plant growth, nutrient uptake and stress-tolerant response due to the humic substances. The differences in the efficacy and other related data obtained in the laboratory and field conditions must be minimized to make biostimulants more popular among the farmers. However, efforts are also needed to harmonize the assessment of biostimulants to identify the inherited product risks and to make efficacy of the products more transparent[53].

Efficient monitoring tools are required to monitor the efficacy of biostimulants. The long-term effects of biostimulants should also be examined. The companies associated with the manufacture of biostimulants must provide agro-ecosystem-based integrated solutions applicable at the field level and help in the decision-making processes. All the stakeholders, including farmers, research organizations, regulatory bodies, etc. should join hands to promote biostimulants in order to enhance sustainability in plant production and profitability for the farmers. Harmonization of policies and regulations to develop a robust risk assessment procedure is required. Appropriate steps are also needed to avoid duplication of data requirements across regulations. Biostimulants may serve as efficient tools for sustainable crop production by modifying the physiological processes of plants to make them tolerant against abiotic and biotic stress under varying climatic conditions.

At policy revel certain areas in Indian regulation of biostimulants like relaxation on data requirement on toxicity, mixture of products, crop grouping based label claim, pooled data generation, avoiding use of animals for tox data by using available alternatives, fortification based on scientific evidences, use of microbes and microbial products as biostimulants, limit of pesticides in products, tolerance limit of ingredients and monitoring and evaluation of products needs proper attention to facilitate the use of biostimulants in the country.

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