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Biostimulants for Sustainable Crop Production: A Review

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Abstract
Green revolution has been made the so many impacts on agricultural production, soil health, environment and ecology positively and negatively. Now so many scientific studies are evolved to reduce the negative effect of green revolution like residual effect of pesticide, fertilisers and synthetic chemicals. In that biostimulants are the important one, they influence the plant growth, productivity, quality of product, nutrient uptake and nutrient assimilation. This review article gives the applications of biostimulants with its research evidences.

Keywords: biostimulants, sustainable agriculture, crop production, nutrient uptake, stress tolerance, environmental sustainability, soil health, plant growth, agricultural practices.

Introduction

Now a days farmers are like to follow modernized agriculture at the same time they won't allow soil detoriation and mostly they prefer ecofriendly farming activities. After the Green revolution inorganic chemical fertilizers and pesticides use was gradually increased. Because of this soil health, environmental surroundings, products quality, human and animals' health are detoriated and End users (consumers) have more preference for food without toxic residues or low toxic residues. Farmers also have burden to produce their products environmentally safe and sustainable in nature. Therefore, primarily Farmers needs the alternative to chemical inputs. In this condition biostimulants are best bio active compounds and part of the viable alternative to inorganic inputs. So biostimulants are gained from raw organic materials *(*Bhupanchandra *et al.*, 2020*)*. Biostimulants have greater capability to change the physiological processes and they quickens the plant's growth, development and contingency response and adaptations (Du Jardin P, 2012). This review paper objectives to provide the whole story and recent information on the advanced developments to make use of biostimulants to improve the crop productivity, product quality, nutrient uptake especially nitrogen uptake and assimilation and abiotic and biotic stress tolerance. Modern agriculture faces the challenge of meeting the growing global demand for food while minimizing its environmental impact. Biostimulants offer a promising solution by optimizing plant growth, resource use efficiency, and crop resilience. This review explores the current state of biostimulant research and their potential contributions to sustainable agriculture.

Biostimulants:

According to the European Biostimulants Industry Council (EBIC, 2012), plant biostimulants are substances and/or micro-organisms whose purpose, when applied to plants or the rhizosphere, is to stimulate natural processes, leading to improved nutrient uptake, enhanced nutrient efficiency, increased tolerance to abiotic stress, and enhanced crop quality. Yakhin *et al*. (2016) described biostimulants as formulated products of biological origin that enhance plant productivity due to the unique and emerging properties of their constituent components, rather than solely relying on the presence of essential plant nutrients, plant growth regulators, or plant protective compounds.

Classification of biostimulants:

1. **Seaweed Extracts**: Seaweed-based biostimulants are derived from various types of seaweed, such as kelp. They contain a range of beneficial compounds like plant hormones (auxins, cytokinins), amino acids, vitamins, and minerals. These substances can enhance plant growth, root development, and stress resistance.
2. **Humic Substances**: Humic and fulvic acids are organic components derived from decaying plant and animal matter. They improve soil structure, nutrient availability, and water retention. Humic substances can also stimulate microbial activity in the soil, aiding in nutrient cycling.
3. **Amino Acids and Protein Hydrolysates**: Amino acid-based biostimulants provide plants with readily available forms of nitrogen and carbon. They can enhance nutrient uptake, photosynthesis, and stress tolerance. Protein hydrolysates are enzymatically broken-down proteins that supply plants with amino acids and peptides.
4. **Beneficial Microorganisms**: Biostimulants can also contain beneficial microorganisms such as mycorrhizal fungi, rhizobacteria, and trichoderma. These microorganisms can form symbiotic relationships with plants, improving nutrient absorption and protection against pathogens.
5. **Plant Growth-Promoting Compounds**: These biostimulants consist of natural compounds like plant hormones (auxins, cytokinins), vitamins, and enzymes that promote various aspects of plant growth and development, including root growth, flowering, and fruiting.
6. **Plant Extracts**: Some biostimulants are derived from plant extracts other than seaweed, containing specific compounds that stimulate plant growth and stress tolerance. These extracts may come from plants known for their beneficial properties, such as aloe vera or neem.
7. **Chitosan**: Chitosan is a biopolymer derived from chitin, found in the shells of crustaceans. It can stimulate plant defense mechanisms, improve nutrient uptake, and protect against certain pests and diseases.
8. **Silicon-Based Biostimulants**: Silicon-based products, like potassium silicate, can enhance plant cell structure, increase resistance to abiotic stressors (e.g., drought, heat), and deter some insect pests.
9. **Biochar**: Biochar is a type of charcoal produced from organic materials. When added to soil, it can improve soil structure, increase water retention, and promote microbial activity, thereby enhancing plant growth and nutrient availability.
10. **Enzymes**: Enzyme-based biostimulants contain specific enzymes that can aid in nutrient mineralization, breakdown of organic matter, and the overall improvement of soil health.

According to EBIC, Biostimulants are categorized into following categories. They are,

- Non-microbial biostimulant

➢ Microbial biostimulant

I. Non-Microbial biostimulants:

- Organic acids
- Protein hydrolysates and other N- Containing compounds
- Sea weed extracts
- Chitosan and Other biopolymers

II. Microbial biostimulants:

- Mycorrhizal fungi
- Plant growth- promoting rhizobacteria

I. Non-Microbial biostimulants:

Organic acids

Humic acid and fulvic acids are commonly employed as biostimulants, arising as products of microbial decomposition. They exert various stimulating effects on plant growth and development. When humic acid is present in the soil, it interacts with plant roots, resulting in reduced shoot development and hydraulic conductivity while not impacting root growth. This effect is particularly beneficial under conditions of water stress (Asli et al., 2010).

The combination of solid and liquid organic fertilizers with a humic acid spray on cauliflower demonstrates positive effects on plant dry weight, plant yield, chlorophyll content, and the uptake and assimilation of nutrients (Mahmood et al., 2019). Similarly, the prior foliar application of humic acid, in conjunction with pre-sowing seed treatment with zinc, when applied to beans in zinc-deficient soil conditions, leads to higher yields and an increased percentage of protein content (Kaya et al., 2005).

Protein hydrolysates and other N containing compouds:

Protein hydrolysates were defined as ‘mixtures of polypeptides, oligopeptides and amino acids. These are derived from plant and animal derived protein sources by hydrolysis process. Quite a lot of field and green house experimental trials were revealed that the benefits of PHs on plant development, quality of produce, resource use efficiency like nutrient and water and stress tolerance performance. Protein hydrolysates enhance the plant nutrition in positive way because simple complexes like amino acids and peptides mixtures enhance the quick absorption (Ertani *et al.*, 2013). At the same time plant derived protein hydrolysates decreases the nitrogen use efficiency by reducing nitrate uptake. (Ruiz *et al.*, 2000)

The presence of amino acid and peptides in protein hydrolysates to form complex with minerals and increase the bioavailability of trace elements like zinc, manganese and alkaline soils. PHs stimulate the enzymes contributed in the nutrient uptake thus increase the plant nutrition. For example, soil and foliar applied PHs trigger the ferric- chelate reductase activity in roots of tomato by enhance the uptake of iron under alkaline stressed conditions. (Colla *et al.*, 2015). Application of protein hydrolysate with tropical plant extract in lettuce improves the anti-oxidant and ascorbic acid (Cozzolino *et al.*, 2020).As same as in tomato, application of tropical plant extract and PHs rise the total phenols and vitamin-c. (Caruso *et al.*, 2019)

Seaweed extract:

The liquefying of seaweed for agricultural purpose was first patented in the year of 1912. It constitutes macro and micro nutrients, plant growth hormones and Amino acids also. Generally, Seaweed extract increase the chlorophyll level in leaves, germination percentage in seeds and enhance the yield by improving the flowering and seed set. It plays a vital role in in-vitro plant production and plant protection practices against pest and pathogens. Halpern *et al.* (2015). Sea weed extract was gained from Redalgae species *Kappaphyacus alvarezii* were applied as foliar spray in different concentration on soybean. Higher concentration of sea weed extract enhance the all the growth and yield parameters like plant height, No. of pods/ plant, No. of grains/ pod, test weight, straw yield and grain yield. Grain and straw yield were increased 57.49% and 8.76% respectively. Nutrient assimilation, majorly N, P, K and S was amplified in grain by up to 36%, 61%,49%, and 93% respectively (Rathore *et al.*, 2009). Application of sea weed extract on cowpea enhance the vegetative growth, chlorophyll pigments and yield attributes. Leaf area/ plant was increased by 47.91% than control. (Youssef *et al.*, 2019).

Chitosan and other polymers:

Chitosan is the deacetylated form of chitin, a biopolymer that occurs naturally as a component of fungal cell walls, insect exoskeletons and crustacean shells. Chitin and chitosan are co-polymers of N-acetyl- d- glucosamine and d- glucosamine. Chitosan acts on the pathogen. The initial study about anti pathogen activity about the chitosan was published by Allan and Hadwiger in the year of 1979 (Allan *et al.*, 1979).

This chitosan broke the peptidoglycan bond of pathogen's cell wall, this makes the electrolyte leakage it leads to the microbe's death (Malerba *et al.*, 2016). Chitosan has good impact not only on pathogen management. Chitosan used for mass production of predatory insects for biological control. Generally, ladybird beetle (*Propylea japonica*, Coleoptera: Coccinellidae) used as predator of aphid. Chitosan and Ca-alginate used as artificial diet to lady bird beetle it results that improves the predation capacity than liquid artificial diet.(Tan *et al.*, 2015)

Chitosan act as anti-transpirants and avoid the oxidative stress (Khan *et al.*, 2002). Chitosan could be extraordinary material applied to avoid the effect of water stress on the growth and yield of plant and quality of produce by producing various metabolites responsible for low transpiration. (Abu-Muriefah, 2013)

II. Microbial biostimulants:

Plant growth promoting bacteria

The genus *Azotobacter, Rhizhobium and Azospirillum spp* are Plant growth promoting Bacteria.

Table 1. Common modes of action for biocontrol of plant diseases by plant growth promoting rhizobacteria (PGPR)

Mode of action	Mode of action description
Antagonism	Bacteria are capable of producing antibiotics, which are then released in appropriate amounts into the specific regions of the rhizosphere, directly targeting and eliminating pathogens. These antibiotics encompass a variety of compounds, among them hydrogen cyanide (HCN).
Signal interference	Certain plant growth-promoting rhizobacteria (PGPR) have the capability to

	break down homoserine lactones (AHL), which are believed to serve as signaling molecules for specific pathogenic bacteria. AHLs may also play a role in biofilm formation. Therefore, the degradation of AHLs by select PGPRs can influence the capacity of bacteria to function as pathogens.
Induced systemic resistance	The presence of specific plant growth-promoting rhizobacteria (PGPR) in the rhizosphere can lead to the development of resistance in the plant against particular pests and pathogens. In contrast to antagonism, induced systemic resistance does not necessitate the extensive colonization of the root system by PGPR, potentially allowing for quicker and more reliable action against pests.
Siderophore production	In soils with low levels of Fe^{3+}, the production of siderophores by PGPR can lead to the sequestration of iron, making it less accessible to phytopathogens. This, in turn, contributes to improved plant health.
Competition for nutrients and niches	Certain strains of PGPR may have the ability to completely colonize the plant roots, outcompeting pathogens for both nutrients and space. However, whether this phenomenon is consistently observed under field conditions remains uncertain.
Interference with pathogen activity and competition	By establishing growth on or within the pathogen, it disrupts the pathogen's essential growth and functions.

The table 1. Summarized from (Bhupenchandra *et al.*, 2020)

Most of the plant growth promoting bacteria have interaction with plant hormones, so its helpful to under and above ground parts development (Lugtenberg *et al.*, 2009).Various species of PGPR increase the tolerant ability of plant under the both biotic and abiotic stress through production of 2-oxbutanoate and NH_3 by convert the originator for ethylene. (Ahmad *et al.*, 2008)

Inoculation of PGPR like *Bacillus megaterium* and *Bacillus muciaraglaginous* together with AMF into the maize crop enhance the assimilation of nutrients especially N, P and K, And also increase the root shoot weight in cotton (Hemissi *et al.*, 2019).

Mycorrhizal fungi

Mycorrhizal fungi having the activity of biostimulant such symbiosis predominantly Glomus it forms most branched hyphae. This helps to extracts the nutrient and moisture under suboptimal condition and improves the tolerance ability. In general, 60- 70% yield gap was caused by abiotic stress. To escape from this unfavorable effect, using of biostimulant is very helpful. Biostimulant containing AMF+ *Trichoderma*+ rhizosphere symbiotic bacteria application enhances the seed yield and pod yield in Bean under the water stressed condition (Petropoulos *et al.*, 2020). AMF inoculum promotes the nutritional status of tomato plant and also reduce the adverse effect of saline stress by higher the production of antioxidative enzymes. AMF act as Bioprotector (Polo *et al.*, 2006).

Modes of action

Biostimulants exert their effects on plants through various modes of action, as you've mentioned. Understanding these mechanisms is essential for optimizing their use in agriculture and horticulture. Here are the key modes of action for biostimulants:

- **Nutrient Uptake Enhancement (Nutrient Mobilization)**: Biostimulants can improve the availability and uptake of essential nutrients by plants. They may chelate or complex nutrients in the soil, making them more accessible to plant roots. Some biostimulants contain compounds like amino acids that can act as carriers for nutrients, facilitating their transport within the plant. Enhanced root development stimulated by biostimulants can lead to increased surface area for nutrient absorption.
- **Stress Tolerance Improvement**: Biostimulants can enhance a plant's ability to withstand various stressors, including drought, salinity, extreme temperatures, and disease. They may activate stress response pathways in plants, leading to the production of protective compounds like antioxidants. Biostimulants can also improve water retention in the soil, helping plants cope with water stress.
- **Hormone Regulation**: Biostimulants may contain natural plant growth regulators like auxins, cytokinins, and gibberellins. These compounds can influence plant growth and development. By promoting hormone balance, biostimulants can lead to increased root growth, flowering, and fruit set. They can also help regulate ethylene production, which can affect plant responses to stress and senescence.
- **Microbial Activity Stimulation**: Some biostimulants contain beneficial microorganisms (e.g., mycorrhizal fungi, rhizobacteria) or compounds that promote microbial activity in the rhizosphere (the soil region surrounding plant roots). These microorganisms can improve nutrient cycling, solubilize minerals, and protect plants from pathogens. Enhanced microbial activity can lead to improved soil health, which in turn benefits plant growth.
- **Enzyme Activation and Organic Matter Decomposition**: Biostimulants may contain enzymes or compounds that activate specific enzymes in the soil. These enzymes can break down organic matter, releasing nutrients for plant uptake. Improved organic matter decomposition can lead to increased soil fertility and nutrient availability.
- **Improved Plant Cell Structure**: Silicon-based biostimulants, like potassium silicate, can strengthen plant cell walls, making plants more resistant to physical stressors, such as wind and lodging. Enhanced cell structure can also deter some insect pests.
- **Antioxidant Production**: Some biostimulants induce the synthesis of antioxidants in plants. Antioxidants help protect plant cells from oxidative damage caused by environmental stressors.
- **Root Growth and Development**: Biostimulants often stimulate root development, resulting in a larger and more efficient root system. This expanded root system can better absorb water and nutrients from the soil.

The specific mode of action of a biostimulant can vary depending on its composition and the plant species it's applied to. Proper application and timing are crucial for harnessing the full benefits of biostimulants in agriculture and gardening.

Benefits of Biostimulant Application:

Biostimulants provide several benefits when applied to crops, making them valuable tools for sustainable agriculture. Here are the key advantages of using biostimulants:

- **Increased Crop Yields**: Biostimulants can enhance plant growth and development, leading to increased yields. They promote root growth, flowering, and fruit set, which can result in higher crop productivity. Improved stress tolerance and nutrient uptake also contribute to higher yields, especially in challenging environmental conditions.

- **Improved Nutrient Use Efficiency**: Biostimulants enhance the availability and uptake of nutrients from the soil. This leads to better nutrient utilization by plants, reducing the waste of fertilizers. Increased nutrient use efficiency can result in cost savings for farmers and a reduced environmental impact through decreased nutrient runoff.
- **Reduced Reliance on Synthetic Chemicals**: By enhancing plant health and natural defense mechanisms, biostimulants can reduce the need for synthetic pesticides and fertilizers. Reduced chemical inputs contribute to sustainable and environmentally friendly agricultural practices.
- **Enhanced Abiotic Stress Tolerance**: Biostimulants can help plants withstand abiotic stresses such as drought, salinity, extreme temperatures, and waterlogging. Improved stress tolerance allows crops to maintain productivity in challenging conditions, reducing yield losses.
- **Soil Health Improvement**: Biostimulants can stimulate microbial activity in the soil, leading to improved soil health. Enhanced soil structure, nutrient cycling, and organic matter decomposition result in a more fertile and resilient soil ecosystem. Healthier soils promote long-term sustainable agriculture by maintaining soil fertility and structure.
- **Environmental Benefits**: Reduced chemical inputs and enhanced nutrient use efficiency have environmental benefits, including lower pollution of water bodies due to reduced nutrient runoff. Biostimulants can also contribute to reducing greenhouse gas emissions associated with synthetic fertilizer production and application.
- **Better Crop Quality**: Biostimulants can improve the quality of harvested crops. They may enhance color, flavor, and nutritional content. Enhanced crop quality can lead to higher market value and consumer satisfaction.
- **Flexibility and Compatibility**: Biostimulants are often compatible with various agricultural practices and can be integrated into existing farming systems. They can complement other sustainable agriculture practices, such as organic farming or precision agriculture.
- **Crop Resilience**: Biostimulants can help crops recover from stress events more quickly, allowing for shorter recovery periods and less impact on yields.
- **Biological Pest and Disease Management**: Some biostimulants contain beneficial microorganisms that can help protect plants from pests and diseases, reducing the need for chemical treatments.

Challenges and Future Directions:

The use of biostimulants in agriculture has shown promise, but several challenges and future directions need to be addressed for their effective and sustainable adoption:

1. **Regulation and Standardization**: The regulation of biostimulants varies from country to country, and there is a lack of standardized definitions and classifications. Developing consistent regulatory frameworks is crucial for ensuring product quality and efficacy. Establishing clear guidelines for product labeling, testing, and claims will help farmers make informed decisions about biostimulant products.
2. **Lack of Comprehensive Scientific Data**: More extensive research is needed to fully understand the mechanisms of action of different biostimulants and their effects on various crops and environmental conditions. Comprehensive scientific studies can provide a stronger foundation for recommendations on biostimulant use and optimize their application methods.
3. **Variable Efficacy across Different Crops and Environments**: Biostimulants may not work equally well for all crops or in all types of soil and climatic conditions. Understanding the factors

that influence their efficacy is essential. Tailoring biostimulant formulations to specific crops and environments can improve their overall effectiveness.

4. **Environmental and Economic Considerations**: While biostimulants can reduce the need for synthetic chemicals, their production and application can have environmental impacts. Sustainable sourcing of raw materials and environmentally friendly production processes should be encouraged. Farmers need to weigh the economic costs and benefits of biostimulant use, considering factors such as product cost, increased yields, and reduced input costs.
5. **Integration with Other Sustainable Practices**: Biostimulants should be viewed as part of a broader sustainable agricultural strategy. Integrating biostimulants with practices like organic farming, precision agriculture, and crop rotation can maximize their benefits. Understanding how biostimulants interact with other agricultural inputs and practices is essential for optimizing their use.
6. **Education and Extension Services**: Farmers and agricultural professionals need access to education and training on the proper selection, application, and timing of biostimulants. Extension services and outreach programs can play a crucial role in disseminating knowledge about biostimulant use.
7. **Consumer Acceptance**: Consumer preferences for crops grown with biostimulants, organic practices, or reduced chemical inputs may impact market demand. Consumer education and awareness campaigns can influence these preferences.
8. **Long-Term Research and Monitoring**: The long-term effects of biostimulant use on soil health, crop productivity, and the environment require ongoing research and monitoring. Studies on the sustainability of biostimulant practices over extended periods will help assess their long-term benefits and potential drawbacks.
9. **Innovation and Product Development**: Continued research and innovation in biostimulant product development can lead to the creation of more effective and environmentally friendly formulations. Exploring novel sources of biostimulants, such as byproducts of other industries or waste materials, can also be valuable.

Conclusion

Biostimulants helps to achieve an eco-friendly intensive agriculture with higher productivity. Numerous valuable experiments are conducted by many scientists about biostimulants and their interaction with plants and environment, effects of biostimulants etc… According to that pool of reviews biostimulants are long-lasting viable alternative to the inorganic fertilizers and pesticides. Very low concentration of stimulative products enough to cause a huge protection and production effects on crops. It increases the productivity with low cost of cultivation. Simply biostimulants are act as stimuli, it stimulates the naturally existing protective and production factor mechanism in plants. Biostimulants have demonstrated their potential to contribute to sustainable crop production. However, further research and development are needed to fully harness their benefits while addressing existing challenges. By understanding the mechanisms of action and optimizing their application, biostimulants can become integral components of environmentally responsible and productive agriculture.

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