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Role of biostimulants in uptake of nutrients by plants

Anitha KV**Abstract**

Biostimulants have been shown to increase nutrient uptake under certain conditions with appropriate dosage, suggesting their usefulness in reducing fertilizer use without affecting yield. However, biostimulants though readily available in the market place do not significantly reduce fertilizer use in conventional agriculture. It is important to find the most promising biostimulants for specific conditions and the amount and methods for the best application, before they can be effective in reducing fertilizer use.

Keywords: Biostimulants role, nutrient uptake

Introduction

One of the biggest challenges for agriculture is the development of sustainable and environmentally friendly systems to address the need to feed the growing world population. With decreasing area of arable land as we approach the limits of genetic potential of staple crops, the only way to achieve this objective is by increasing the crop yield and protecting what we produce. In other words, produce "more with less". Parallel to this, reducing energy consumption and utilizing resources more efficiently quality of crops should be enhanced, particularly under unfavorable growing environments. This means obtaining higher incomes for farmers, having better postharvest storage and more nutritious food for consumers should be priorities.

Innovative solution

One of the most innovative and promising solutions to address these important challenges consists of the used of plant biostimulants, referred as "materials which contain 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/or crop quality, independent of its nutrient content" by European Biostimulant Industry Council [EBIC], 2016.

According to agro pages, the market of Biostimulants is estimated to worth \$1,402.15 million in 2014, projected to reach \$2,200 million by 2018 and \$2,524.02 million by 2019. The global market for BS is projected to increase 12 % per year. The expected drivers are the growing importance for organic products in the agriculture industry, increase of bio-stimulants application in developing countries and more global bio-stimulants presence and acceptance among customers- innovative products - satisfy specific crop needs.

Bio stimulants for Agriculture

- To supplement and enhance existing agricultural practices and crop inputs.
- Foster plant growth and development throughout the crop life cycle from seaweed extracted germination to plant maturity
- Improve efficiency of the plant's metabolism to induce yield increase and enhanced crop quality
- Increase plant tolerance to and recovery from abiotic stresses
- Facilitate nutrient assimilation, translocation
- Enhance quality attributes of produce *viz.* sugar content, colour, fruit seeding, etc.
- Render water use more efficient Enhance soil fertility, by the development of complementary soil microorganisms.

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According to EBIC, biostimulants distinguish themselves from traditional crop inputs in three ways:

1. Bio-stimulants operate through different mechanisms than fertilizers, regardless of the presence of nutrients in the products
2. They differ from crop protection products because they act only on the plant's vigor and do not have any direct actions against pests or diseases
3. Crop bio-stimulation is thus complementary to crop nutrition and crop protection

Main categories of plant bio-stimulants

1. Basis of source and content
2. Humic substances (HS),
3. Hormone containing products (HCP) like seaweed extracts which contain auxins, cytokinins, or their derivatives
4. Amino acid containing products (AACP).

Commonly used plant bio-stimulants

1. Humic and fulvic acids
2. Amino acids
3. Seaweed extract
4. Plant-growth-promoting bacteria
5. Chitosan and other biopolymers
6. Inorganic compounds

Table 1: Characterization of humic substances

Properties	Humic acid	Fulvic acid
Elemental composition (%)		
C	54-59	41-51
H	3-6	4-7
N	1-4	1-4
S	0.1-0.5	0.1-3.5
O	33-38	40-50
Functional groups		
Total acidity	500-900	700-1400
COOH	150-600	500-1100
Phenolic OH	200-600	100-600
E4/E6 ratio	3-5	6-9
Molecular wt.	10,000-2,00,000	100-1,000
Polymerisation	Longer chain, high	Long chain but less
Aromaticity	High	Low
Colour	Brown	Yellow

1. Humic Substances (HS)

Humic substances are heterogeneous organic molecules that form in the soil as byproducts of microbial metabolism of dead organic matter. Humic substances are one of the most common organic substances on Earth and make up 60% of the organic matter in the world's soils. Humic substances can be extracted from many different sources, including soils, municipal waste, vermicomposts and earthworm casts, various coal deposits, peat and Leonardite. Humic substances can be applied to the plant in a number of ways, including foliar applications, in the irrigation water and direct application to the soil.

Effects of humic substances on plant growth

Humic Substances have a number of positive effects on plant growth, including increased biomass, increased number of fruits and flowers, and improved fruit quality.

Effects of humic substances on nutrient uptake

There have been a number of studies showing that humic substances increased NO₃ uptake. Plant acquisition of each of

these nutrients was affected differently by different doses; some doses affected certain minerals positively and others negatively greatly improve phosphorus and iron uptake in maize when they are applied to soils with little organic material. When applied to soils with high concentrations of organic material, the positive effects were small or nonexistent, probably because the background levels of humic substances were already high in those soils. Some studies found positive effects of humic substances on micronutrient uptake, specifically in alkaline soils or alkaline nutrient solutions where micronutrients are often limiting.

Mechanisms by which humic substances affect nutrient uptake

Humic substances improve plant nutrition by affecting soil processes and by directly affecting the plant's physiology. The mechanisms that affect the soil processes include:

1. Humic substances improve soil structure

Humic substances improve plant nutrition by improving the soil structure, amending the soil with humic substances increases aggregate stability. Humic substances has the ability to form clay-humic complexes with hydrophilic components oriented toward the center of the aggregate and hydrophobic components facing outward. This reduces water infiltration into the aggregates, making them more stable in wetting and drying cycles. Improved aggregate stability leads to improved soil aeration, facilitated root penetration, greater water availability to the plant, and less soil erosion, which indirectly contribute to enhanced nutrient uptake. However, improved aggregate stability does not explain the observed improvement in plant nutrition in hydroponic systems, or when the humic substances are applied to the foliage rather than the soil.

2. Humic substances improve solubility of micronutrients and P

Under some circumstances, micronutrients and P are highly insoluble. Humic substances added to the nutrient solution enhance Fe and Zn solubility by forming metal-humic complexes. Application of the water-soluble fraction of humic substances increased the solubility of Fe-hydroxides, as well as their mobility in the soil. Humic substances have been shown to be effective replacements for artificial chelates of Fe such as ethylene diamine-N, N0-bis (2-hydroxyphenylacetic acid) (EDDHA) in tomato, lemon trees, and grapevines grown in calcareous soils. Humic substances also increased the activity of plasma membrane H⁺-ATPase which could lead to rhizosphere acidification and hence to increased solubility of micronutrients. Humic substances increased the availability of phosphorus by interfering with the formation of nonsoluble Ca-phosphates. This explains the increased efficiency of P use when soluble phosphate fertilizers are applied to soils that have been amended with organic materials.

3. Humic substances change root morphology

The auxin-like activity of humic substances, stimulates plasma membrane H⁺-ATPase, thereby stimulate cellular growth. An increased in lateral-root and root-hair development increases the surface area of the root, which would explain the increased nutrient uptake induced by humic substances.

4. Humic substances stimulate H⁺ ATPase and NO₃-assimilation enzymes

Auxin-like activity of the humic substances and nitric oxide signaling, by stimulating the PM H⁺ATPase, Humic substances acidify the rhizosphere, causing the NO₃ H⁺ symport system to work more effectively. The cumulative result is that the plant absorbs more NO₃. Humic substances also increase the rate of NO₃ assimilation by causing the plant to upregulate the enzymes involved in this process. Humic substances derived from compost have a stimulatory effect on many NO₃-assimilation enzymes, including nitrite reductase (NR), glutamate synthase (GS) and aspartate amino transferase.

Application of vermicompost on FYM „N“ equivalent basis resulted in higher grain and haulm yield of soybean and also higher nutrient uptake. Further, the crop receiving soil application of humic substances (humic and fulvic acid) @ 5 kg ha⁻¹ at sowing + foliar application of humic substances (humic and fulvic acid) extracted from vermicompost (0.2%) at 40 DAS helps for better nutrient uptake by the crop and to obtain higher yield in soybean (Savitha *et al.*, 2018) [6].

2. Amino Acids (AA)

Amino Acids are a large family of biological compounds that contain an amine functional group and a carboxylic acid functional group. There are only 20 amino acids involved in protein building, but there are 250 more that are known to have diverse functions in plants, including protection from biotic and abiotic stresses, signaling, N storage and chelation of metals as phytosiderophores. Commercially available amino acids biostimulants are mostly mixtures of different amino acids and short peptides, rather than pure substances. These mixtures, called protein hydrolysates, are derived from the hydrolysis of proteins from plant, animal and microbial sources, often from industrial and agricultural waste products such as crop residues, animal skin, feathers and blood. Protein hydrolysates are marketed as plant biostimulants that can be applied as a foliar spray, soil drench, or seaweed extracted treatment.

Absorption of Amino acid by plants

Plants can absorb amino acids directly into the roots when they are dissolved in the mass flow of water into the xylem through specific transporters in the roots or via diffusion into the leaves. Plants can utilize amino acid as a source of N and under some circumstances, in certain plants, amino acid are the main source of N.

Effects of Amino acid on plants

Amino acid application has been shown to increased biomass production, help protect plants against biotic and abiotic stresses and increased the antioxidant content of the leaves.

Effects of Amino acid on nutrient uptake

Application of exogenous amino acid to plant leaves and roots has been shown to increase nutrient uptake and nutrient-use efficiency for both macro and micronutrients

Mechanisms by which Amino acid affect nutrient uptake

amino acid application can improve plant nutrition by affecting soil processes and by affecting the plant's physiology directly.

A. Amino acid increased soil microbial activity

Amino acid application to the soil increases soil microbial activity, which can improve the soil's physical and chemical attributes. Specifically, the increased bioactivity in the soil causes quicker breakdown of organic matter, which transforms organic nutrients into plant available mineral forms.

B. Amino acid chelate micronutrients

Amino acid can chelate metals such as Fe, Zn, Mn, Cu making them more readily absorbable through the roots and leaves via specific transporters, such as LHT1. In nature, plants often secrete specific nonprotein amino acids known as phytosiderophores from their roots into the soil to improve micronutrient availability.

C. Amino acid reduce micronutrients

Specific amino acids may also increase the availability of micronutrients by acting as a reductant and exogenous application of cysteine to maize roots in a hydroponic solution causes an increase in Cu uptake. It is hypothesized that the cysteine acts as a reductant, changing Cu II to Cu I, which may be more available to the roots.

D. Amino acid improve internal translocation of micronutrients

Amino acid chelates are also important for the translocation of micronutrients within the plant. There have been a large number of studies showing that nicotianamine, a nonprotein amino acid, is responsible for the translocation of micronutrients in the phloem. Nicotianamine has also been shown to have positive effects on plant physiological processes, even when applied exogenously, and it can therefore be considered a biostimulant. Specifically, exogenous application of nicotianamine increased the translocation of Zn and Fe to the grains of rice plants, which has important implications for human nutrition.

E. Amino acid affect root morphology

Exogenous application of amino acid has also been shown to have an effect on root morphology. Specifically, L-glutamate application to the root inhibited primary root growth and stimulated root branching. It also stimulated root-hair development close to the root tip.

F. Amino acid stimulate NO₃-assimilation enzymes

Amino acid have also been shown to stimulate the NO₃-assimilation enzymes through hormonal action. The enzymes measured in the root and leaves included NR, NiR, GS, and GOGAT.

Plant growth was improved by the municipal soil waste compost as compared to the others. Comparatively, the vermicompost had the highest amount of amino acids and mineral nutrients followed by the seafood waste compost. The municipal soil waste compost, seafood waste compost, and vermicompost were composed of total of 36.4, 48.3, and 67.5mg amino acids/100 g dry weight, respectively. Glutamic acid, aspartic acid, and glycine were the highest while methionine, histamine, and cysteine were the least in all the amendments. The vermicompost had the highest Ca content but the least P and K contents. Seafood waste compost had the highest content of P and K and most of the determined micronutrients (Lord Abbey *et al.*, 2018) [4].

3. Seaweed Extract (SE)

Seaweed extract a weed has been applied as a fertilizer in coastal regions for centuries. The first method for liquefying seaweed extract a weed for agricultural use a weed extract was patented in 1912. Liquefied seaweed extract is usually manufactured from *Ascophyllum nodosum*, a brown seaweed extract a weed that is commonly found in the North Atlantic, although other species, such as *Durvillaea antarctica*, *Durvillaea potatorum*, *Macrocystis pyrifera*, and *Ecklonia maxima* are also used as weed extract.

A. Seaweed extract improves soil structure

Seaweed has been applied as a fertilizer in coastal regions for centuries. The first method for liquefying seaweed for agricultural use was patented in 1912. Liquefied seaweed extract is usually manufactured from *Ascophyllum nodosum*, a brown seaweed that is commonly found in the North Atlantic, although other species, such as *Durvillaea Antarctica* and *Ecklonia maxima* are also used.

B. Seaweed extract affects root-to-shoot ratio

Application of seaweed extract to either leaves or roots has been shown to lead to an increase seaweed extract in root mass or root-to-shoot ratio in various different crops.

C. Seaweed extract promote symbiotic relationship between mycorrhizal fungi and roots

Arbuscular mycorrhizal fungi are associated with the roots of most terrestrial plants, and they play an important role in nutrient uptake. Seaweed extract has been shown to encourage arbuscular mycorrhizal fungal growth and infection rates.

Foliar spray of 10 per cent seaweed sap extracts of *Kappaphycus alvarezii* and *Gracilaria* sp. with 100 per cent recommended dose of fertilizers could improve nutrient content in index leaves, cane and nutrient uptake by sugarcane (Leindah Devi and Mani, 2014)^[3].

4. Plant-Growth-Promoting Bacteria (PGPB)

Group of root colonizing bacteria, that stimulate the plant growth. Plant growth promoting bacteria can be inoculated to seaweed extracted or directly into soil and are usually mixed with a carrier material such as peat, manure, compost. The seaweed extract carrier provides favourable environment for the Plant growth promoting bacteria.

Direct influence: Seaweed extract solubilization and uptake of nutrients or production of plant growth regulating substances.

Indirect effect: Pathogen suppression by production of siderophores or antibiotics

Mechanisms by which plant growth promoting bacteria affect plant nutrition:

There have been many studies showing the positive effects of plant growth promoting bacteria on plant nutrition.

A. Some plant growth promoting bacteria Fix N

One of the earliest plant growth promoting bacteria mechanisms discovered was N fixation and commercial inoculations of N-fixing Rhizobia, which form symbiotic relationships with legumes, have been available since the 1890s. Mixed inoculations of endophytic diazotrophic bacteria such as *Gluconacetobacter diazotrophicus*,

Burkholderia tropica, *Azospirillum amazonense*, *Herbaspirillum rubrisubalbicans*, and *Herbaspirillum seropedicae* have also been shown very effective at promoting N fixation. In fact, many free-living N-fixing plant growth promoting bacteria which were thought to improve plant growth because of their ability to fix N have since been shown to promote plant growth through other mechanisms.

B. Some plant growth promoting bacteria solubilize P

Some plant growth promoting bacteria have been shown to improve plant nutrition through phosphorus solubilization. The total concentration of phosphorus in agricultural soils usually ranges between 400 and 1200 mg kg⁻¹. However, only 1 mg kg⁻¹ is generally present in available forms such as HPO₄⁻ and soils is present in inorganic and organic forms. The nonsoluble inorganic forms account for about 20–50 % of the total soil phosphorus (Richardson, 2001)^[5], usually in the form of PO₄⁻ ions. Bacteria use a number of strategies to solubilize the nonsoluble inorganic and organic phosphorus compounds. To solubilize inorganic phosphorus, bacteria often synthesize organic acids such as gluconic and citric acids, which chelate the insoluble compounds and lower the pH, both of which increase phosphorus solubility. Another mechanism is to simply release protons, which lowers the pH and increases solubility without the help of chelates. Bacteria also increase phosphorus availability by mineralizing organic phosphorus. The ability to solubilize phosphorus is common in rhizospheric bacteria and many such bacteria have been isolated, including those from the genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Enterobacter*, *Streptomyces*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium*, and *Erwinia*.

C. Some plant growth-promoting bacteria promote a symbiotic relationship between mycorrhizal fungi and roots

One of the most interesting mechanisms by which plant growth promoting bacteria enhance plant nutrition involves promotion of the symbiotic relationship between mycorrhizal fungi and plant roots. The plant growth promoting bacteria that promote this relationship are called mycorrhiza helper bacteria. Many plant growth promoting bacteria have been found to promote mycorrhizal fungal growth, including *Agrobacterium*, *Streptomyces*, *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Burkholderia*, *Arthrobacter*, *Azospirillum*, *Klebsiella*, *Azospirillum*, *Alcaligenes*, *Rhizobium*, *Bradyrhizobium* and *Brevibacillus*.

Treatments with *Pseudomonas putida*, B 17 and B 19 almost doubled the grain iron content. Besides this, the translocation efficiency of the iron from roots to shoots to grains was also enhanced upon treatment with plant growth promoting rhizobacteria. The presented results show that the application of several plant growth promoting rhizobacteria strains not only led to an increase in iron uptake by plants but also increased the translocation of iron into the grains It is therefore concluded that application of plant growth promoting rhizobacteria strains is an important strategy to combat the problem of iron deficiency in rice and consecutively in human masses (Shrama *et al.*, 2013).

5. Chitosan and other Biopolymers

Chitosan is a deacetylated form of the biopolymer chitin, produced naturally and industrially. The physiological effects of chitosan oligomers in plants are the results of the capacity of this polycationic com-pound to bind a wide range of

cellular components, including DNA, plasma membrane and cell wall constituents, but also to bind specific receptors involved in defense gene activation, in a similar way as plant defense elicitors.

6. Inorganic compounds

Chemical elements that promote plant growth and may be essential to particular taxa but are not required by all plants are called beneficial elements. The five main beneficial elements are Al, Co, Na, Se and Si, present in soils and in plants as different inorganic salts and as insoluble forms. Many effects of beneficial elements are reported by the scientific literature, which promote plant growth, the quality of plant products and tolerance to abiotic stress. This includes cell wall rigidification, osmoregulation, reduced transpiration by crystal deposits, thermal regulation via radiation reflection, enzyme activity by co-factors, plant nutrition via interactions with other elements during uptake and mobility, antioxidant protection, interactions with symbionts, pathogen and herbivore response, protection against heavy metals toxicity, plant hormone synthesis and signaling.

In poor soil and when nutrition is deficient, use of biostimulants containing humic acid, amino acids, algae extract, and microelements could improve yield and quality of lettuce plants (Karima *et al.*, 2018)^[2].

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