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Bio-Stimulants- An Overview

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ABSTRACT: Bio-stimulants are the formulated products of biological origin, which are used to increase the crop growth, plant height, increase the yield and to reduce the infestation of pests and diseases in the specific crop. These product shows the positive responses within the crop, the quality parameters in these products are absolutely meet the requirements given by the approval board and hence certified by the Standing committee on chemicals and fertilizers. The Bio-stimulants does not differ much from the Plant Growth Regulators (PGRs). These are products with similar, but heightened abilities to that of PGRs. These Bio-stimulants increases a plants resistance towards Abiotic stresses within the environment.These Bio-Stimulants faces major challenge in marketing as their approval as a widely used products is still not considered by farmers. Bio- Stimulants contribute to low input, high output and sustainable crop productions. In this review article I have discussed the functions, sources, classifications, applications in various crops, and problems faced in market approval.

Keywords: Biological origin, standing committee on chemicals & fertilizers, PGRs, Abiotic stress, plant height, NUE and yield increase.

INTRODUCTION

"A plant Bio-stimulant is any substance or microorganism, in the form in which it is supplied to the user, applied to plants, seeds or the root environment with the intention to stimulate natural processes of plants benefiting nutrient use efficiency and/or tolerance to abiotic stress, regardless of its nutrient content, or any combination of such substances and/or microorganisms intended for this use" (Def. Proposed by ARCADIA)

The bio-stimulants are those products which reduces a plants requirement of nutrients and fertilizers. By the application of Bio-stimulants the plant uses the low available nutrients efficiently, so as that there is no requirement of fertilizer application. These Bio-stimulants serves a great purpose and ensures the agricultural sustainability in those areas which possess agricultural lands with less availability of nutrients. These products when applied at low concentrations are pretty much beneficial to the plant but, when applied in high concentration there will be noticeable fatality responses shown by the plants (Carolina *et al.,* 2019). the Bio-stimulants are not useful in barren lands where there is no fertility and minimum available nutrient in the soil. These products have gained a lot of audience (farmers) and has been gathering attention since past $2^{1/2}$ decades from both commercial and scientific communities (Crouch and van Staden, 1993; Herve, 1994; Zhang and Schmidt, 1999; Maini, 2006; Khan *et al.,* 2009; Apone *et al.,* 2010; Craigie, 2011; Sharma *et al.,* 2014; Brown and Saa, 2015; Du Jardin, 2015; Yakhin *et al.,* 2016; Yakhin *et al* 2017).

The plant Bio-stimulants was named under different synonyms over the past 30 years based on their origin, and mode of action (Lefort, 2021). It was first experimented on sugarcane crop under moderate water stress conditions which results in rise in rates of photosynthesis, transpiration, and stomatal mechanisms. The 'Bio genic' stimulant theory is initially given by Prof. V.P. Filatov in USSR in the year 1933 (Filatov, 1944, 1951; Gordon, 1947; Sukhoverkhov, 1967; Yakhin *et al* 2017). He proposed that the environmental stresses can cause both metabolic and enzymatic effects in plants, humans, and animals. The works are further carried by Blagoveshchensky in the years 1945, 1955, 1956. He considered the Bio genic stimulants as organic acids due to their properties to increase the enzymatic activity in plants.

Basic Functions of Bio-stimulants:

1. Root structure and growth – The application of these biological substances helps in the formation of good root structure and stimulates growth.

2. Water use efficiency – The biological substances reduce the water need of a plant by utilizing the less water to complete their metabolism.

3. Nutrient use efficiency – Bio-stimulants mitigates the crops need for fertilizers by utilizing the nutrients provided to the crop scarcely.

4. Stress tolerance – Bio-stimulants mainly concentrates on increasing the tolerance of the crop to the environmental stresses caused by both Biotic and abiotic factors.

5. Disease resistance – These organic compounds aid the crops against the regular and lethal diseases by the increasing a plants disease tolerance ability.

CATEGORIES:

Although there are different types of bio-stimulants, only 2 of them are popular kinds of bio-stimulants which are based on the substance, and micro-organisms. The substances include Humic and fulvic acid, seaweed extracts, protein hydrolysates, inorganic compounds, chitosan. The micro-organisms include the rhizospheric, epiphytic, PGRs, beneficial fungi, beneficial bacteria and endophytic microbiota are also being domesticated for the future application (Lefort, 2021). The bacteria, fungi and PGRs can befree-living, rhizospheric or endosymbiotic in nature (Du Jardin 2015). Among these ingredients the acid-based materials acquire the largest Bio-stimulant market followed by Seaweed based materials (Ute Albrecht, 2019).

Classification Based on Substance

Humic and Fulvic acids: - (Du Jardin. P. 2015; Du Jardin. P. 2012; Ute Albrecht, 2019).

These are heterogenous compounds, categorized based on their solubility and molecular mass from the Humic substances (HS). These humic substances are of the natural origin as per the reason, that they are accumulated from the plant, animal decays, along with soil microbial residues. The humic acid is darker in colour and high molecular weight, whereas Fulvic acid is lighter in colour and possess low molecular weight. Humic acid also consists of high degree of polymerization and carbon content.

The Humic substances are resulted due to interactive relationship between plant roots, organic matter, and microbes. For someone to use any of these humic substances for the beneficence of the plant growth, they must analyze and interpret this interactive relationship.

The Humic substances are obtained from the sources like humidified organic substances (like cocopeat, volcanic or organic soils) and mineral depositions (e.g., soft coal and leonardite), which are Non-renewable sources and can also be obtained from renewable and sustainable agricultural sources like compost and vermicompost.

"Humic like Substances" obtained from the controlled breakdown and by the oxidation through chemical process are proposed as an alternate for the natural humic substances. (Du Jardin 2012; Ute Albrecht, 2019). There are 2 derivatives of humic acid which are known as plant derived humic acids PDHA and Coal derived humic acids CDHA (Khan *et al.,* 2016).

Humic acid is an environmental product since it is obtained through decomposition of organic matter, and aid in plant growth by chelation of unavailable nutrients in the soil, their uptake and buffering pH (Mackowiak *et al.,* 2001). The polyanionic nature of the humic substances results in increased soil cationic exchange capacity which aid in the nutrient uptake through roots, Biological, physical, and physiochemical attributes of soil.

Seaweed extracts: The seaweed extracts are used for agricultural practices for over thousands of years (Calvo *et al.,* 2014) and has recognized to be used as a Bio-stimulant in 1950's. the seaweed extracts are reported to act as chelators, which improves the utilization of and uptake to mineral nutrients by plants provides aeration which stimulates the root growth. (Milton 1964). Majority of the commercial seaweed extracts are prepared from brown seaweeds, including Ascophyllum nodosum, Fucus, Laminaria, Sargassum, and Turbinaria spp (Hong *et al.,* 2007; Sharma *et al.,* 2012).

The seaweed extracts also provides the plants with required resistance to withstand against both biotic and abiotic stresses. (Zhang *et al.,* 2016). The seaweed extracts come in various forms like powder and liquid form based on the mode of extraction. (Battacharyya *et al.,*2015). The commercial extract was manufactured by using the methods which includes water, acids, alkalis as extractants, these are used either by heating or without it, and physical disruption of seaweed using low temperature milling and high pressure (Herve and Rouillier 1977; Stirk and van Staden 2006; Craigie 2011).

The seaweed extract is in the form of liquid, and it is applied to the plants along with the fertilizers and micronutrients (Milton 1862, Craigie 2011). If available in large quantities (seaweed extracts) can be applied with irrigation. The mixture of seaweed extracts may vary according to the time or season of collection and the method or extractants used for the process of extraction (Khan *et al.,* 2009; Rioux *et al.,* 2009; Sharma *et al.,* 2011; Shekhar *et al.,* 2011). Seaweed extracts have numerous effects on plant metabolism (Khan *et al.,* 2009).

(Jannin *et al.,* 2013) studied the effect of application of the seaweed extract on gene expression in genotypes of Brassica Napus plant. It was found that 1000 genes were showing differential gene expression in microarray analysis. Out of 1000 genes, most affected gene were related to photosynthesis, cell metabolism, sulfur metabolism, nitrogen metabolism and response to stress tolerance.

The seaweed extracts are observed to alleviate the suffering of plant caused by the abiotic stresses like Drought, salinity, and extreme temperatures (Nabati *et al.*,1994; Zhang and Erwin 2004; Mancuso *et al.*, 2006; Khan *et al.*,2009; Craig *et al.*, 2011; Calvo *et al.*, 2014). Seaweed extracts contain a wide range of plant hormones like auxins, gibberellins, Cytokinin's, abscisic acid, salicylic acid (Khan *et al.*, 2009; Craigie 2011). The seaweed brown algae have been used for the agricultural purposes since 13th century without any complications (Temple and Bomke, 1988).

Protein Hydrolysates: The protein hydrolysates are derived from sources like crop residues, animal wastes, and through industrial byproducts like collagen, leather, and epithelial tissues. These protein hydrolysates consist of amino acid and peptides that are acquired through Agro-chemical processes involving the sources. There is a special case derivative of amino acids called as "Glycine betaine" which is known to possess the ability to induce resistance in plants to the stress caused by the heavy metals (Chen and Murata, 2011; du Jardin, 2015; Ute Albrecht, 2019).

Protein hydrolysates affect the metabolisms of the crop indirectly by increasing the growth and productivity through increased soil fertility, microbial biomass, soil respiration, and soil microbial activity. The special case amino acids and peptides are considered to induce the available soil nutrient uptake through roots with greater rate (Calvo *et al.*,2014; Ute Albrecht, 2019).

Protein hydrolysates can alter the nitrogen uptake and its assimilation through roots by altering the gene structure and regulating the nitrogen assimilation caused by the enzymes of TCA (tricarboxylic acid) cycle. Some protein hydrolysates possess chelating effects like Proline which helps in the acquisition and transportation of micro-nutrients inside the plant and protects them against stress caused by the heavy metals (Du Jardin, 2015; Ute Albrecht, 2019; du Jardin, 2012).

Proline and glycine are well known protectants which enhances the natural resistance in the plants against environmental stresses like drought, salinity, etc. the protein hydrolysates of animal origin are deemed as harmless, the bioassay tests involving plants and yeast as test subjects reported that there was no trace of toxicity involving genetic, ecological, and phytological origins (Corte *et al.*,2014).

Chitosan's: Chitosan's are the polymers or oligomers that are used in the food, cosmetics, medicine and established their status recently in Agriculture. they possess the ability to bind to a receptor which is held accountable for the activation of plant defenses (*Disper Bio-stimlants*). These compounds have been classified as Natural Agro-chemicals as they are useful in seed coating, fruit and vegetable preservation, growth promoter, and fungicide (Freepons 1997; Assis and Pessoa, 2004; Allan and Hadwiger 1979; Burrows *et al.*, 2007; Velasquez *et al.*, 2012).

Chitosan's are the derived form of Chitin which are produced both industrially and naturally. They come in various sizes based up on their field of application. Other than binding to the defense receptors, these polycationic compounds also binds to DNA, plasma membrane and cell wall constituents (Du Jardin, 2015; El Hadrami *et al.*, 2010; Hadwiger, 2013; Katiyar *et al.*, 2015; Yin *et al.*, 2010).

The use of chitosan's in recent agricultural works has increased drastically due to their offered protection against the fungal pathogens and induced tolerance to abiotic stress factors like, cold, heat, drought, salinity, etc. These compounds are obtained through extraction processes involving fungal cell wall, prawn outer part or crab shell. chitosan's induces stomatal closing through Abscisic acid mechanism (Iriti *et al.*, 2009; du Jardin, 2015).

(Darren 2019), These compounds not only help in the stress tolerance and enhancing plants own defense mechanism, but also obviously aid in pest tolerance, inhibition of micro-organisms growth, and environment friendly due to its microbial degradation and non-toxic nature. Hence, its role in agriculture is considered as green production. Chitosan's is known as soil conditioners, because their addition in soil can increase the beneficial bacteria like actinomycetes and remove harmful bacteria like fusarium from the soil.

Classification Based on Micro-organisms

Beneficial Fungi: Fungi which possess the activity of bio-stimulants belongs to a group called symbiotic fungi. These symbiotic fungi intertwine with the plant in many ways from mutualism to parasitism (Behie and Bidochka, 2014). Mycorrhizal fungi maintain symbiosis in over 90% of the plants available in nature, and in these fungal forms the Arbuscule-Forming Mycorrhiza (AMF) is widely spread and maintains association with both agricultural and horticultural crops,This AMF comes under the category of endo-mycorrhiza(Bonfante and Genre, 2010; Behie and Bidochka, 2014; Du Jardin 2015). The AMF not only helps in nutrient uptake, but also aid in the phosphorous uptake especially in phosphorous deficit soils (Ute Albrecht, 2019).

The fungi belonging to the species Glomeromycota forms a Branche-tree like structure called abuscules by infiltrating the root zone of a plant, thus allows the increased nutrient uptake by extending the roots deep into the soil. Some endophytic fungi like *Trichoderma spp.* and *Sebacinales* had the ability to completes a portion of their life cycle independent on the host, forms the root structure deeply in a way to enhance the nutrient uptake by the host and are well distinguished from mycorrhizal fungi (Bonfante and Genre, 2010; Behie and Bidochka, 2014; Du Jardin 2015).

Recent studies have proven that the hyphal network of the plants not only establishes a symbiotic relationship between fungi and plants but also interconnects a plant with another individual plant in their vicinity. This provides significant ecological evidence that symbiotic

fungi contribute for the interplant signaling (Johnson and Gilbert, 2015; Simard *et al.*, 2012; Du Jardin 2015).

Beneficial Bacteria: Unlike beneficial fungi, the beneficial bacteria do not restrict its relationship with plants with mutualism and parasitism, in fact they use every possible opportunity to interact with the plant. (Ahmad *et al.*,2008).The Bacteria which are isolated from soil, water, plants, compost, manures, and plant residues came to known as Microbial inoculants (Berg 2009; Dodd and Ruiz-Lozano 2012; Vessey 2003;).

The beneficial bacterial are bio-control microorganisms (BCMs) which are today known as "Bio-stimulant Microorganisms" helps in the plant growth and offer protection to them right from seeding stage to crop maturity against harmful pathogens. The bacterial can not only travel through soil but also transmitted vertically through seed (Duanyin *et al.*, 2015; Adriano, *et al.*, 2014).

Based on their utilization in agriculture as bio-stimulants they can be categorized into 1.) mutualistic Rhizobium: - they are commercial Bio-stimulants as they can participate in Nutrient assimilation through plants. These rhizobium symbiosed bio-stimulants are often seen in the textbooks of even elementary standards due to their extensive agricultural usage (Du Jardin 2015; Ahmad *et al.*, 2008; Babalola, 2010; Berendsen *et al.*, 2012; Berg *et al.*, 2014; Bhattacharyya and Jha 2012; Gaiero *et al.*, 2013; Philippot *et al.*, 2013; Vacheron *et al.*, 2013).

2.) Rhizospheric PGRs: - often known as "Plant growth promoting rhizobacteria" is one of the main Beneficial bacteria formulations, which possess the capacity to increase the mineral uptake, root growth, NUE, morphogenesis, response to Biotic and Abiotic stresses within the plants intermediate locations known as rhizosphere and rhizoplane (Du Jardin 2015; Duanyin *et al.*, 2015; Nguyen, 2014).

Applications in wheat: -

Humic substances in wheat: The humic acid plays an important role in wheat production. It increases the major attributes in the wheat crop like, retention in fertilizer usage, nutrient uptake, high yield, root vitality, and improved seed germination percentage. (Anwar *et al.*, 2016). Humic substances have the capacity of single superphosphate to provide phosphorous to the plant when combined with the low-concentrated phosphate rock. This single superphosphate (SSP) provides plant with high nutritional uptake value, and this value can be compared to the combination of Humic acid and Araxa phosphate rock. This combination results in good wheat growth (Rosa *et al.*, 2018).

(Anwar *et al.*, 2016) Humic acid can be applied solely or with nitrogen and phosphorous (NP) fertilizers in case of rainfed conditions to ensure optimal production. The humic acid has limited effect on wheat attributes in comparison with the nitrogen fertilizers applied to the soil. For the organic wheat cultivation, Humic acid application is commendable, and is also effective against drought stress. Application of humic acid @ 2% concentration is proven to effectively increase the grain yield by 26% and straw yield by 23.8% and can also be increased by increasing the rate of application of humic acid by 15 kg/ha and nitrogen by 150 kg/ha.

Seaweed extracts in wheat: When seaweed applied**,** wheat crop stimulates various growth parameters during the early stages of the crop and decreases the senescence, increase the metabolism rate, and performs assimilates redistribution during the reproductive stages of crop development. To optimize these effects, the seaweed concentrate known as Kelp66 is applied to the crop at early vegetative stage and should be applied at correct concentration. If applied at low concentration it doesn't stimulate growth, whereas when applied high concentration the crop should good growth but does not provide optimum kernel yield. (Nelson and Staden 1986).

Chitosan's in wheat: Defang Zeng, Xinrong Luo conducted experiment in wheat crop under drought stress condition. The application of chitosan coating to the seed increases the Rate of germination, length of the root, wet weight, root activity and impacted some physiological indices like MDA (malondialdehyde). The chitosan application increases the antioxidant activity thereby decreasing the MDA content to reduce the damage caused by drought stress and improve seedling growth. The chitosan's also increases the chlorophyll content which is responsible for the photosynthetic activity and results a spike of 13.6% in yield when compared to CK applied seeds, (Zeng and Luo 2012).

Protein hydrolysates: The foliar application of chicken feather protein hydrolysates (CFPH acquired through alkaline hydrolysis process) in wheat seeds has proven to increase the physiological activity, biochemical activity and stimulate the root length, shoot length, dry weight of wheat seedlings. In addition to these parameters, a spike in photosynthetic pigment content is also observed. From these we can conclude that chicken feather protein hydrolysates can be used as a Bio-stimulants which yields good results (Genc and Atici 2019).

Applications in Rice:

Humic substances in Rice: The humic acid treatment results in the larger root, increased root weight and good shoot growth in the early phases of crop growth. The treatment of humic acid given during the metaphase increases the number of grains per head and high percentage of ripened grains. The treatment also markedly increases the respiration rate in paddy root irrespective of growing period. Furthermore, application in early phase of growth yield increased nutrient uptake in stub whereas, in later phase there is a significant difference (Isao and Jinya 1988).

Seaweeds extracts in rice: Rice is the most staple crop in India. India has the largest land to produce rice (say

approx. 44 million hectares) next to China. The most popularly used bio-stimulant in rice is seaweed extract liquids, as they show promising results over the recommended dose of fertilizer. The use of seaweed Bio-stimulants increases the panicle height, panicle weight, grains per panicle, grain yield and harvest index. The seaweed extracted Bio-stimulants also help in the growth of meristematic, translocation of photosynthates, enzyme activation, cell elongation and cell stability (Arun *et al.,* 2019; Pramanick *et al.,* 2013). The increase in yield is mainly due to the efficient utilization of nutrients present in the soil, induced nutrients through roots and foliar application of seaweed extract liquid. There is an evident recording of higher yield, improved nitrogen, total soluble solids and rise in Oryzein when application is done at 30- & 60-day interval (Haider *et al.,* 2012).

Chitosan's in rice: The applications of 0.1% chitosan solution in non-fertilized soil before plantation yield in increasing shoot growth, plant height and number of leaves which is not observed in fertilized soil(Chibu*et al.,* 2002). The chitosan application affects the physical properties of the rice like- the rice noodles when subjected to chitosan solution, the moisture remains unaffected whereas the pH and the white color of the texture is decreased. Storing after application at 30°C for 5days resulted in an increase in the hardness, gumminess and decrease in the cohesiveness, and springiness of rice flour gel (Klinmalai *et al.,* 2017).

Applications in Maize:

Humic substances in maize: Cultivation of maize with Humic acids redounded in advanced fresh weight of roots than in undressed shops (control). Shops treated with Humic acids showed increased number, periphery, and length of roots. Cytoskeleton proteins, similar as tubulin (tubulin beta- chain) were over- regulated in the sludge roots treated with Humic acids. Lower situations of tubulin protein result in a hypersensitiveness response at high salinity. hence, cytoskeleton proteins may be important in guarding the factory against swab stress. Protein folding- related proteins were only detected in maize roots treated with Humic acids; these include proteins similar as calnexin, calreticulin and endoplasmin- resembling protein (Rosane *et al.*, 2019).

Chitosan's in maize: (Tawaha *et al.,* 2018): conducted two experiments in maize crop; 1. To observe the effects of Chitosan's on two varieties of maize to relieve the salinity stress and 2. To observe how different kinds of Chitosan's affects the maize crop under salinity stress. At the end of the experiment, he concluded that the salinity stress shows negative effect on the root and shoot growth by decreasing the weight of root and shoot, reducing plant height and leaf size in both varieties of the maize. Foliar application of Chitosan's has resulted in better growth of plants in non-salinity conditions and alleviated the severe effects of maize grown in saline conditions. It's also concluded that transpiration rate is affected by neither salinity nor Chitosan application.

(Sharma *et al.,* 2020), the seed treatment and foliar application of Chitosan nano fertilizer comprising of copper and salicylic acid influences increasing the source activity in maize development. Seed treatment induces high seed vigor, while the foliar application with nano fertilizer increases Antioxidant enzyme activity and amplify the chlorophyll content in leaves. In the internode region the Sucrose translocation is induced by foliar application which allows to study the enhanced mobilization of nutrient towards growing cob.

Seaweed extracts in maize: Foliar application of 20% Seaweed extract (*Sargassum crassifolium*) can enhance the vegetative growth of maize seedlings. The characteristics that are recorded to be improved are leaf area, plant height and dry matter accumulation (Sutharshan *et al.,* 2017). Foliar application of seaweed extract (Goemar GA14) to maize seedlings increases the fresh matter production by 15 to 20% over control. This yields a noticeable result due to increased root and stem mas per plant. After two weeks of spraying there is less significant difference in stomatal resistance and carbon assimilation rate. However, the physiological mechanism of this Goemar GA14 and its mode of action in plants have not yet elucidated (Jeannin *et al.,* 1991*).*

Problems: Two of the larger challenges facing the trade are: Associate unsure restrictive atmosphere in some geographies and market saturation in others. There has been a boom in investment and interest during this house in some countries and as a result there's an intense competition for the similar markets. The major problems faced by the Bio-stimulants products in India: as we know that the farmers of Indian nation are poor and can merely afford its pricing. So, not many of the farmers are up for buying the products. hence, they stick to the old, regional, and religious techniques to grow the crop.

CONCLUSION

By the above provided information on the Bio-stimulants, we can conclude that they play a vast role in sustainability of agriculture by mitigating the use of chemical fertilizers. Bio-stimulants enhances the enzymatic activity in the plants. They aid in the good performing of plant's vital processes thus, increase the yield. They help in the production of good quality products. They are applied for the soil-nutrient enrichment, provides stability to the crops, Increases the nitrogen use efficiency (NUE) and reduces the need for fertilizers, Tolerance towards abiotic stress conditions and incase of sugarcane it helps in Maintenance of higher rates of photosynthesis, transpiration, and stomatal conductance. We can conclude that the seaweed extracts are pretty effective in terms of application and are highly utilized by natural course of application. The global market for

bio-stimulants is projected to increase 12 % per year and reach over $2,200 million by 2018. (Duanyin *et al.*, 2015) but, currently its growth was reached to 3.2 billion dollars in 2021 and is estimated to reach 5.6 billion dollars by 2026 (Dublin, 2021). The Bio-stimulants definition and formulations are undergoing change from day to day which explains the increasing demand for its useful effects in improving yield and grain quality in different crops (Carolina and Helena, 2019).

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