

Bio-stimulants: Concept, Types, and way to enhance Soil Health

Abstract

Soil has immense capacity to function as a vital living ecosystem that not only supports plants, and animals, also acknowledges survival of people is referred as Soil Health. There has been renewed interest in soil health due to the environmental repercussions of poor management, such as soil erosion and nutrient contamination. As a result, bio-stimulant application can have a significant impact on soil health. Bio stimulants are compounds, microorganisms or other materials capable of stimulating nutrition processes in plants or in their growth environments. Regardless of their nutritional content they increase the plant's nutrient use efficiency, partial factor productivity, tolerance to abiotic stress and quality of the crop. Many types of biostimulants have been differentiated by their administration technique either soil or foliar, or may be plant or animal derivatives, or by the distinctive procedure involved for their derivation that may be hydrolysis, fermentation or acid/alkali extraction. Stimulants of biological origin that are soil applied can promote the establishment, proliferation of beneficial soil organisms that furnish substrates for plant growth. The use of environmentally friendly natural preparations is especially significant in light of the ongoing processes of soil degradation and air pollution. Enzymes, Protein hydrolysates, and Sea Weed Extracts, Humic substances, Nitrogen-Fixing Bacteria and Phosphorus Solubilising Bacteria, Plant Growth Promoting Rhizobacteria briefly comes under the umbrella of bio stimulants. Even while a bio stimulant may not have a short-term effect, it has the potential to improve soil health with progression, ensuing higher yields in the succeeding years.

Keywords: Bio stimulants, Humic substances, Soil biota, Soil Health

1. Introduction

To accomplish contemporary food demand, agriculture relies heavily on nutrients suppliers of synthetic origin (fertilizers) and chemicals for pest control (pesticides), intensified tillage operations, and sufficient irrigation water, which grounds and serve as base for the loss of vital ecosystem services, pollution and high greenhouse gas emission (Kopittke et al., 2019). As an upshot, biotechnologies that enable effectual resource management particularly of water, nutrients, and soil while assuring elevated yields and polished agro based products are the need of the hour and will prove to be decisive in the near future for agricultural intensification to be sustainable (Petersen et al., 2015). Developing innovative technologies and tactics to increase the partial factor productivity and resource use efficiency especially of water and fertilisers as well as to augment productivity of fields crops both under optimal and

suboptimal conditions which is at most critical to safeguard food security while focusing on conserving quality and health of soil in accordance with providing ample opportunities for farmers (Jagermeyr et al., 2020). Chemical fertilizers application is a cost effective and efficient technique of providing mineral nutrients to crops in a short span of time frame but they are habitually washed off the field in runoff or might become inaccessible to crops (Chen, 2006; Halpern et al., 2015) and may cause various environmental issues and even influence human health. Also, the industrial manufacturing of chemical fertilisers influences the energetics of the system as its manufacturing is a highly energy-intensive procedure (Dzikurrokhim, 2021) that has been linked to hefty boost in global CO₂ emissions (Brightling, 2018). Organic fertilisers, such as compost, sludge, or manure, offer the assistance of utilising nutrients that are before now present in the Agro-ecosystem and requiring modest energy input to be processed, mineral nutrients bound in organic materials may be supplementary stable, and consequently less likely to be washed away or discharged into the atmosphere. but the constriction with organic nutrient sources is they don't provide crops with nutrients easily in water-soluble form which is absorbed easily compared to insoluble forms. Also, the supply is not in synchronised form i.e., supply of nutrient when the crop requires them (De Pascale et al., 2017; Timsina J., 2018). One way to offset this shortcoming is to cultivate such crops which have a more robust root systems and comparative better nutrient-uptake efficiency, ensuring that they get hold of nutrients when they are needed, despite of their condensed immediate availability when delivered organically. Nutrients can also be made additionally accessible by encouraging specific sort of organisms within the soil microbial population. Both of these techniques may be accomplished by addition of bio stimulants to crop leaves, seeds, or soil to fuel up root development (Halpern et al., 2015). Biocentric products, for instance biostimulants, is a sustainable, competent technique or supplement to their mock (synthetic) equivalents for enhancing NUE (nutrient use efficiency) coupled with yield solidity of agricultural and horticultural crops in optimal and sub-optimal state of affairs. (Fiorentino et al., 2018). A diamond cut technology would be the application of natural plant stimulants (PBs), which can enhance flowering, plant growth, fruit development, crop output, and nutrient utilisation efficiency, to raise crop resilience to a wide range of abiotic stressors and improve agricultural productivity (NUE). The exploitation of biostimulant chemicals can advance nutrient absorption and assimilation to a great extent which has been proven by several studies on greenhouse and open-field vegetables. Increased plant nutrient absorption has been endorsed to one or more of the subsequent factors: increased soil enzymatic and microbial

activity, transformation in root architecture, and improved micronutrient mobility and solubility (De Pascale et al., 2017). The foundation of extracts of biological origin (biostimulants) is assorted, being obtained from diverse organic sources, for instance microbial fermentation of animal or vegetable raw materials, industrial residues, humic substances, algae extracts, protein hydrolysates, beneficial fungi and rhizobacteria that sponsor plant growth (Rodrigues et al., 2020). Other substances, chiefly synthetic and not extracted from organic biological resources, may have stimulating properties but are not so far considered as plant biostimulants (Soppelsa et al., 2018; Rodrigues et al., 2020). Plant biostimulants are the most time and again used term for some precise goods intended to stimulate crop development; even so, other labelling names for these commodities include biofertilizers, plant probiotics, bio stimulators, and metabolic enhancers (Sible et al., 2021). The endeavour of this review article is to build up a basic, indispensable concept and understanding about biostimulants, their major cataloguing and highlighting the after math of bio stimulant application especially on soil health.

2. Concept of Biostimulants

Prof. V.P. Filatov commenced the foremost debate of "biogenic stimulant" hypothesis in the Soviet Union in 1933. Filatov hypothesised that biological components that were brought into being from stress-exposed species (including plants) that might influence various energy processes and impact metabolic activity in humas, animals, and plants (Filatov, 1944; Yakhin et al., 2017). Herve, presented the primary factual conceptual approach to biostimulants, he proposed that the development of biostimulants which he gave the term - "bio-rational products" should be based on an orderly sequential method which would utilise the science of chemical synthesis, biochemistry, and biotechnology approaches applied to actual plant keeping in mind the physiological, agricultural and ecological limitations. He believes that these products should be used at modest doses, also should be ecologically friendly and possibly deliver proven benefits in agricultural plant production system (Herve, 1994; Yakhin et al., 2017). A systematic dialogue on biostimulants was established by Yakhin, the conceptual groundwork for the current biostimulant science (Basak, 2008; Yakhin et al., 2017). Biostimulants utilised in field crops are expended to lift crop yields and the nutritional content the result of which is that they are frequently being used in agricultural management strategies targeting at curtailing chemical inputs, improving productivity, and restoring natural agro-ecosystem balance (Woo et al., 2018).

The new Regulations by (EU) 2019/1009, delineates that plant biostimulants as follows : “EU fertilizing product, the function of which is to stimulate plant nutrition processes independently of the product’s nutrient content with the sole aim of improving one or more of the following characteristics of the plant and/or the plant rhizosphere: nutrient use efficiency, tolerance or resistance to abiotic stress, quality characteristics and availability of confined nutrients in the soil or rhizosphere”. Some believe that biostimulants are nutrient sources but they are not nutrients intrinsically, instead they assist the uptake of nutrients from the rhizosphere by the crop or they may aid in constructive growth advancement or may aid in development of resistance by plants to various biotic and abiotic stresses (Brown, 2015). Plant biostimulants comprise of a comprehensive array of natural constituents as well as synthetic or natural compounds which are chemically derived , on top of valuable microorganisms, which may be categorised as (i) Humic substances; (ii) vegetative or animal-based protein hydrolysates; (iii) macro and micro-algal extracts; (iv) silicon (v) arbuscular mycorrhizal fungi (AMF) and (vi) several plant growth-promoting rhizobacteria’s (PGPR) belonging to the genus *Azotobacter*, *Azospirillum* and *Rhizobium* (Roupael et al., 2020). The Compound Annual Growth Rate (CAGR) of biostimulants was 10.65% till 2019 which proves biostimulant especially plant biostimulant based commerce to be the fastest growing agro industry for now. Whereas comparing the worldwide inorganic fertiliser market, which is presently 60 times larger, is expanding at a CAGR of 1.3–1.8% per annum (Fortune Business Insights Report, 2020). Since not all of the cited parts or components are "biological" in origin and hence the word "bio"-stimulant is rather confusing. The term "bio" denotes to the components of living organisms and their natural ingredients. Non-organic variables, on the other hand, may be regarded as synergistic influencer of numerous “biological” processes that administers plant physiology, metabolism, morphology, and interactions within the agroecosystem (Woo et al., 2018).

3. Categorization of Biostimulants

There has not been any divergent cataloguing of Biostimulants in agriculture. The mode of action of biostimulants and the origin of its active ingredient has been propositioned as a basis on which it could be classified (Basak et al., 2008). Configuration of biostimulant should be the least significant indices for cataloguing them into different groups, instead the categorization should be based on their action in the plants or on the physiological plant responses (Bulgari et al.,2015). Instead of focusing on the characteristic or distinctiveness of

their components or on their modes of action, any definition or categorization of biostimulants should stress on the agricultural functions of biostimulants (Du Jardin, 2015). The following sorting has been extensively acknowledged as;

3.1 Plant biostimulants of Microbial Origin

These Microbial derived plant biostimulants may be progressed with fungi, bacteria, and arbuscular mycorrhizal fungi (Fiorentino et al., 2018). To impersonate the structured biological networks in innate soils, a novel methodology would be "rhizosphere engineering" which proposes the cumulative effect of inoculants of microbial nature, which not only helps in stimulating the revival of functional and beneficial microbial groups certainly linked to fertility of the soil but also in refilling the natural microbiome which has been exhausted by crop domestication (Woo et al., 2018). Plant biostimulants of microbial origin or simply microbial inoculants group predominantly include Plant Growth Promoting Rhizobacteria (PGPR) and endophytic fungi such as AMF and *Trichoderma spp* (Rouphael et al., 2017; Pascale et al., 2017). In recent years, as a result of industrial fabrication of enzymes through microbial fermentation methods, the purification and production of enzymes that can be utilised in cropping systems through soil application are now being industrialised at mass level (Nielsen et al., 2007).

3.2 Plant Extract based Biostimulants

By examining for such species which have protective properties and exhibit high content of antioxidants, an escalating number of biostimulants are being obtained from this knowledge through various extracts of terrestrial plants (Del Buono et al., 2021). Plant extract based biostimulants are protein hydrolysates (PHs) and these hydrolysates are a mixture of oligopeptides and polypeptides as well as of free amino acids which have been developed either by chemical or enzymatic or by chemical-enzymatic hydrolysis of plant residues (Schaafsma et al., 2009; Caruso et al., 2019). The occurrence of osmoprotectants, like sugars and proline in plant extract based biostimulants, is very crucial and of immense importance since these osmoprotectants can sponsor the capacity of crop plants to cope with various biotic and abiotic stress (Sairam and Tyagi, 2004; Del Buono, et al., 2021). Free amino acids and polypeptides containing products of biological origin (plant based) which are obtained through extraction and/or enzymatic hydrolysis are broadly accompanied under plant extract based biostimulants (Zulfiqar et al., 2020). Plant biostimulants especially protein

hydrolysates and natural plant-based extracts those are derived from tropical regions are being extensively used for their beneficial effects on crop productivity and nutritional efficiency (Caruso et al., 2019).

3.3 Seaweed Derived Plant Biostimulants

Fairly low expense of production and the substantial ability of seaweed derived biostimulants to promote considerable increment in the plant biomass makes them the most predominant class of biostimulants in the arcade. The extracts derived from sea weeds can far and widely diverge with seaweed species that may be brown, red or green, temporospatial (occurring in both time and space) origin of the raw material, and practices concerned in attaining the desired biostimulant (Sharma et al., 2014). By and large, the mainstream of PBs obtained from sea weeds originate from brown algal species, to be more precise, from *Ascophyllum nodosum*. The most commonly utilised approach for fabrication of SWEs is Alkali Extraction (sodium or potassium solutions), with or without heating (Craigie, 2011; Stirk et al., 2020). It is critical to turn seaweeds into liquid extracts or soluble powders since dried seaweeds have a gentle decomposition rate i.e., decompose slowly and have an inhibiting effect on plant development due to the generation of poisonous sulfhydryl chemicals that can last up to 15 weeks if applied as such (Stirk et al., 2020). The most commonly utilized seaweed species include *A. nodosum*, *Macrocystis pyrifera*, *Ecklonia maxima*, *Ecklonia radiata*, *Durvillaeapotatorum*, *Durvillaea antarctica*, *Laminaria digitata*, *Laminaria* species, *Sargassum* species and *Fucus serratus* (Sharma et al., 2011; du Jardin, 2015; Stirk et al., 2020).

3.4 Protein Hydrolysate Derived Plant Biostimulants

Protein hydrolysates are another well-known class of PBs that are being comprehensively researched with the aim to help plants cope with various biotic and abiotic challenges. This class of PBs dealing with protein hydrolysates demonstrates the capacity to improve agricultural output and quality, particularly when plants are grown in areas with limited water availability. (Du Jardin, 2015). Protein hydrolysates that are extracted mainly through partial hydrolysis are basically mixtures of polypeptides or of their simpler forms such as

oligopeptides and amino acids (Schaafsma, 2009), and are available in various forms ranging from soluble liquid extracts soluble powder form to solid granular form. These hydrolysates may be either side-dressed near the root zone of the plant or applied as foliar sprays (Colla et al., 2015a). From the wide range of both animal and plant biomass, protein hydrolysates can be produced through various hydrolysis processes such as acid hydrolysis, alkaline hydrolysis, thermal hydrolysis and enzymatic hydrolysis (Colla et al., 2015a; du Jardin, 2015; Halpern et al., 2015). Amino-acids and peptides mixtures are certain compounds acquired by chemical and enzymatic protein hydrolysis from agro-industrial by-products, from both plant sources (crop residues) and animal wastes (e.g., collagen, epithelial tissues) (du Jardin, 2015). The treated species with protein hydrolysates showed boosted carbon and nitrogen metabolism, enhanced nutrient availability and uptake by the crop, and ultimately the nutrient use efficiency (Colla et al., 2017).

3.5 Humic Substances Derived Plant Biostimulant

Plant Biostimulants can be obtained from organic carbon centred products such as manures, compost, etc. by hydrolysis reaction (Tejada et al.,2011). The beneficial effect of humic acid application on plants is achieved by the induction of physiological and structural changes in roots and shoots, allowing plants to better digest and disperse nutrients (Canellas et al., 2015). Obsolete interpretations of this process imply that as an end result, soil organic matter (SOM) contains certain stable chemical compounds collectively known as humus or humic substances. These Humic substances constitute of recalcitrant chemicals which are resistant to further breakdown. These recalcitrant molecules are commonly classified as humic substances (Lamar ,2020). Understanding of average molecular mass and mass distribution (determined using Ultracentrifugation, Sedimentation Velocity, Equilibrium Sedimentation and Archibald approach) is important characterisation of humic substances (Jones and Bryan, 1998). The humic substances can be extracted from various sources (manures, compost, soil, water etc) by several methodologies like alkali hydrolysis, acid hydrolysis,

Seaweed Extracts, Humic and Fulvic Acids, Nitrogen Fixing Bacteria, Phosphorus Solubilizing Microorganisms (PSM), Arbuscular Mycorrhizal Fungi, PGPR, Enzymes, and Biochar fall under the major categorization of Biostimulants (du Jardin, 2015; Sible et al., 2021; Nanda et al., 2021).

4. Impact of bio stimulants on Soil Health

The consequences of application of distinct bio stimulants to soil health (physical, chemical, biological properties) are

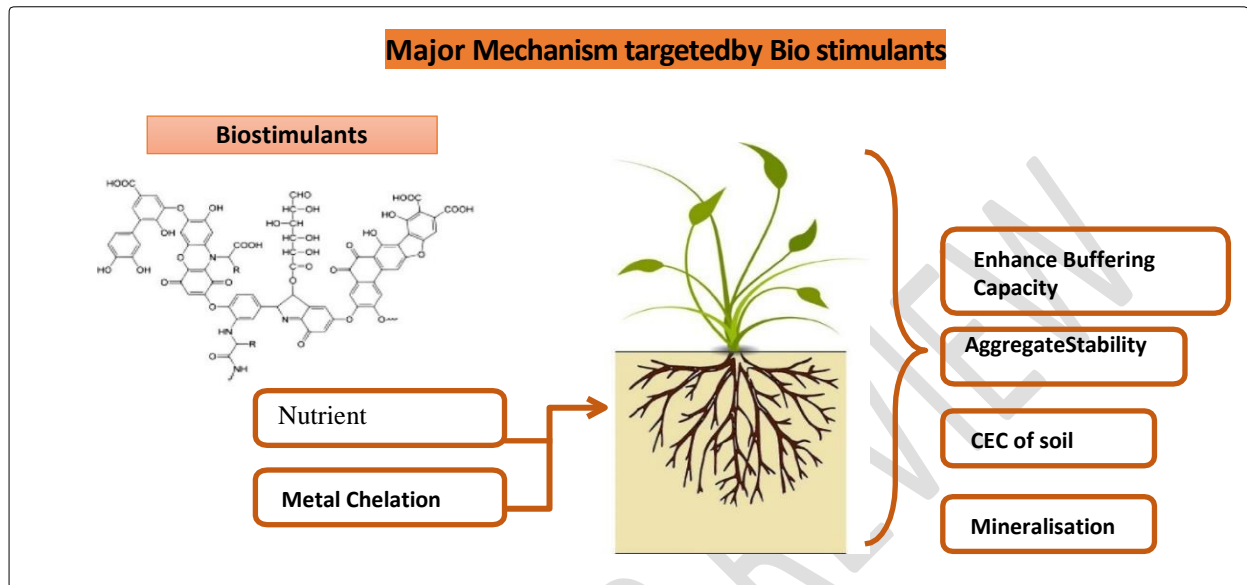


Fig. 1: Major mechanism followed/targeted by bio stimulants

Briefing of Effect of Bio stimulant application to soil

Bio stimulant	Extraction /Isolation method used	Soil characteristic	Effect on soil	source
Humic acid	Leaf compost with 0.5 l NaOH solution (1:5) through 24-hour agitation and precipitation with 6NH ₂ SO ₄ up to pH 2	Clay loam	Addition of Humic acid increased the CEC of soil, because of addition of cations will increase them in the mineral surface and between minerals. reduction of soil pH because of replacement of the soil solution of salt ions with H ⁺ ion	Ali et al., 2018
Commercially formulated Humic acid	Not specified	pH :7.84, clay loam	Amend plant nutrition by intensifying the uptake of N, P, Mg, and Ca thus affecting grain yield. In addition, Humic acid enhances Fe and Zn uptake.	Nasiroleslami, et al., 2021
SWE- <i>Durvillaea potatorum</i> and, <i>Ascophyllum nodosum</i> based	Alkaline hydrolysis	Loam	Increased bacterial proliferation, available nitrogen content in the soil. Bray-Curtis analysis verified SWE treatment created diverse and distinct soil populations. Metagenome analysis disclosed a surplus of microbial communities in the rhizosphere.	Hussain et al., 2021
SWE <i>Ascophyllum nodosum</i> based	- Not specified (Readymade SWE was used)	Loamy sand	Increased abundance, activity and diversity of soil biota. Nectriaceae dominated the fungal communities while Bacterial-root communities were dominated by Streptomycetaceae, <i>Phingomonadaceae</i> , <i>Rhizobiaceae</i> and <i>Pseudomonadaceae</i> .	Renaut et al.,2019
SWE <i>Lessonia flavicans</i> based	Pressurized Liquid Extraction	sandy loam	The activity of urease, proteinase, and phosphatase compounded. based on <i>Lessonia nigrescens</i> and <i>Lessonia flavicans</i> .T-RFLP analysis showed increased abundance of fungal community.	Wang et al., 2016
SWE <i>Macrocystis pyrifera</i> based	Soft Extraction (traditional method)	haplic luvisol	Augmented hydrogenase activity (mg TPF/10 g) in soil by 32% .	Onet et al.,2018
Inoculation with	Isolated on a nitrogen free (NF) -	Not Specified	Augmented the physicochemical, biological properties of soil and also	Harindintwali et

CNFB	CMC/Cellulose agar medium		increased the SOC (Soil Organic Carbon)	al.,2020
Inoculation with PSB	NBRIP for Isolation and acclimation	Shandong brown soils	Compounded richness of bacterial community at both phylum and genus level, Proteobacteria dominated at genus level while <i>Cellvibrio</i> at species level. Transformation of insoluble tricalcium phosphate into soluble phosphorus takes place and this is manifested by polyphosphate kinase gene (<i>ppk</i>) and pyrroloquinoline quinone (<i>pqq</i>)	Wan, W. et al.,2020
Biochar	Pyrolyzing bamboo at 450°C.	Clay loam and sandy loam	Soil Sol-K and Ex-K, soil pH (more in clay loam), AN, AP and total bacterial abundance increased in response to biochar application.	Wang, I. et al., 2018
Biochar	Slow pyrolysis of Eucalyptus spp. bark at 350 °C.	Sandy Loam	Biochar upstretched water retention and the micropore/macropore ratio and reduced bulk density, in addition to improving fertility.	Tanure, M. et al.,2018
Inoculation with <i>Bacillus myloliquefaciens</i> (PGPR)	Isolated from the field soil of Chonnam National University, South Korea	Soil, sand, vermiculite, and compost @ 2: 1: 1 : 0.04 (v/v/v/v), respectively	Enzymatic activities in soil revealed a highly noteworthy interaction with bacterial inoculation, as chitinase and dehydrogenase activities were found to increase.	Jama et al.,2018

Table 1 : Briefing of Effect of Bio stimulant application to soil

4.1 Humic and Fulvic Acids:

Understanding the fundamental system, how plant responses to the biostimulants application, is a significant bedrock for the practical applicability of humic substance in the fields. For successful application the foremost step definitely will be an enhanced understanding of the consequence of humic substance application on various biological cycles such as carbon and nitrogen cycles which are directly correlated to primary metabolism of plant (Olk et al., 2018; Jindo et al.,2020).

Parameter	Fulvic Acid	Humic Acid
Carbon	40–50	50–60
Hydrogen	4–6	4–6
Nitrogen	1–3	2–6
Oxygen	44–50	30–35
Sulphur	0–2	0–2
Molecular Weight	1000–10,000 Daltons	10,000–100,000 Daltons

Table. 2: Chemical composition of Humic and fulvic acids (Sible et al., 2021)

The enhanced carbon (C) content of these organic molecules may serve up as a undeviating energy source for a variety of soil biota., consequently encouraging their activity and potentially ensuring more productive soil (Valdrighi et al., 1996; Sible et al., 2021). Humic and fulvic acid appliance has proven its worth in enhancement of soil's structure via amplified stability of aggregates (Piccolo et al., 1997; Sible et al., 2021). Humic acid, which may be obtained from either Sulfur-enriched leonardite or may be extracted from various sources, has led to establishment of moderate drought-stress tolerance and increased phosphorus availability to plants, ensuring comparatively superior yield when applied before sowing as pre-plant soil amendment (Kaya et al., 2020). Humic acid application leads to increase in soil nitrogen and phosphorus availability to crops, which has also guided to succeeding increase in plant nitrogen uptake, which designates accelerated soil biochemistry in relation to nutrient cycling as a result of soil-applied Humic acid (Sarir et al., 2005). The release of protons and exudates, which is guided by plant roots have illustrated multifaceted dynamics of association or dissociation of these humic substances into supra-molecular

colloids. As Humic substances have stimulating action, root nourishment is advanced through diverse methods, it obstructs the calcium phosphate precipitation, consequentially leading to an intensified macro-and micronutrient absorption, as well as proliferation in the availability and uptake of phosphorus. A very imperative character in soil conditioning and plant growth is played by an active component of organic humus, which is humic acid (Bendetti, 1996; Fahramand et al.,2016). Humic substance application from physical perspective sponsors better soil structure and enhances the water retention capacity of the soil; from biological view, it promotes the growth, activity and abundance of beneficial soil organisms, while chemically it assists in terms of adsorption and retention complex for inorganic plant nutrients (Fahramand et al.,2016). The activity of plasma membrane H⁺-ATPases is encouraged by addition of humic substances, which is involved in the conversion of free energy produced by ATP hydrolysis into a transmembrane electrochemical potential which is further engaged in the import of nitrate and subsequent nutrients into the root system. Breeding broadens electrochemical gradient by ATPase induction and hastening the nutrient uptake rate are the principal mechanisms and these mechanisms can also be authorised by the overexpression of the transporter genes (Jindo et al., 2016; Zanin et al., 2018; Nunes et al., 2019). The Nutrient uptake capability of plant from soil solution can be encouraged not only by root proliferation and nutrient absorption enhancement but also by chelation which progresses the availability of micronutrients such as iron with humic acid application (Aguirre et al., 2009; Zanin et al., 2019).

4.2 Sea Weed Extract:

The application of sea weed grounded biostimulant improves the environment for soil biota development, hence contributing to the improvement of the rhizosphere microbiome. For example, soil treatment of *Ascophyllum nodosum* extract revealed enhanced bacterial biodiversity in the pepper plant's rhizosphere (Renaut et al., 2019; Nanda ,2021). Further, the seaweed extracts comprise a wide range of minerals and chemicals that operates as chelating agents and assist soil health benefits (Khan et al., 2009; Nanda et al., 2021). Application of Sea weed extract adds to soil gel formation, water retention, and soil aeration through their polysaccharides, which are found in soil (Mahusook et al.,2021). In supplementing to improve soil structure and texture, the seaweed extracts also aim to enhance soil aeration and moisture retention capacity of soil (Mukherjee and Patel, 2020). Once such seaweed extracts are applied to soil, the alginates found in it makes complex polymers by edging to metal ions

in the soil by natural chelation process (Battacharyya et al., 2015). Constructive effects via the soil microflora are also pronounced, with the advancement of plant growth-promoting bacteria and pathogen antagonists in suppressive soils. The alginates found in the seaweed extract bond to metal ions in the soil and then they form complex polymers and assist in the natural chelation of soil. Much similar to the chemical fertilizers or pesticides, the seaweed extracts can be applied onto soil or plants directly or as a foliar spray (du Jardin, 2015). The most common method of extraction of components utilised by industries is alkaline hydrolysis, subsequently the other techniques include acid hydrolysis, water-based, microwave-assisted, ultrasound-assisted, enzyme-assisted, supercritical fluid, and pressurized liquid extractions (Shukla, 2019). The macroalgae fluctuate from different organic-based products in their high richness of distinctive carbohydrates present in it, explicitly fucoidan, alginate and laminarin, which are copious in brown algae, carrageen in red algae, and ulvan derived from green algae, all of which are potent biostimulators (Craigie et al., 2011; Sible et al., 2021).

When *Lessonia nigrescens* and *Lessonia flavicans* based sea weed extract was applied to replant soil of *Malus hupehensis* seedlings, a potential increase in the soil activity of invertase, urease, proteinase, and phosphatase enzymes was critically observed. The same findings were substantiated by T-RFLP analysis which exposed that the soil fungal community has been considerably transformed after seaweed extract application to soil (Wang et al., 2016; Ali et al., 2021).

4.3 Arbuscular Mycorrhizal Fungi

Majority of plant species (about 90%) have symbiotic relationships with a mycorrhizal fungus. (Varma et al., 2020) It is one of the numerous forms and taxa of endomycorrhiza allied with crop and horticultural plants, in which fungal hyphae enter root cortical cells and arbuscules are produced (Bagyaraj et al., 2021). Several individuals believe that mycorrhiza can assist in promoting sustainable agriculture for the reason that the benefits mycorrhiza offers in terms of water balance, nutrition efficiency (for both macronutrients and micronutrients, notably phosphorus) and plant protection from biotic and abiotic stresses (Huang et al., 2021). As an outcome of Arbuscular Mycorrhizal Fungi, nutrient levels are more stabilised, and the soil system is more proficient. Biological and abiotic components in soil are interdependent and Arbuscular Mycorrhizal Fungi hyphae form a connection between the two components through the plant's roots inside the pedosphere (Chai et al., 2018) As soil

aggregates are formed, Arbuscular Mycorrhizal Fungi hyphae assist regulate water inflow, improve pore space, and check erosion by constructing a thick network. Microbial-based PB, containing AMF and *Trichoderma koningii*, irrespective of water regimes, amplified in lettuce P, Mg, Fe, Mn, and Zn by 20.8 to 97.4%, the content of various phenolic compounds, and plant yield (Saia et al., 2019; Del Buono D, 2020). AMF symbiosis is particularly important for enhancing the uptake of the relatively immobile and insoluble phosphate ions in the soil, due to interactions with soil bi- and trivalent cations, principally Ca^{2+} , Fe^{3+} , and Al^{3+} (Rouphael et al., 2015).

4.4 Nitrogen Fixing Bacteria, Phosphorus Solubilizing Bacteria and PGPR

When prokaryotes fix nitrogen in the environment, they condense molecular nitrogen to ammonia, which is then absorbed into amino acids. Nitrogen fixation process is mediated by nitrogenase enzyme. There are three distinct classes of nitrogenase enzyme complexes that diverge in their metal cofactor, these are: iron-iron (Fe-Fe), molybdenum-iron (Mo-Fe), and vanadium-iron (V-Fe) (Zehr et al., 2003; Sible et al., 2021). In supplementary to being one of the utmost used bacteria for BNF, *Azospirillum brasilense* has been comprehensively scrutinised for its ability to supply plant hormones (Steenhoudt et al., 2000). Nitrogen-fixing bacteria in the soil saturate it with inorganic N-containing compounds, which are essential crop nutrients. When Nitrogen fixation bacteria dies, the added nitrogen in their biomass is liberated into the soil. Along with this, these bacteria are accountable for the secretion of polysaccharides, the consequences of which results in better aggregate stability and crumbly structure of the soil (Cordeiro et al., 2019).

Only 0.1% of the total phosphorus is offered for the plant, unluckily, the surplus is unutilized by the plant or not available in the soil solution pool. (Sharma et al., 2013) Application of Phosphatic Biostimulants like Plant Solubilizing bacteria (PSB) can assist in making it available through mineralization of organic-P pools or solubilization of inorganic phosphates. Most of the inorganic phosphates are solubilized by bacteria as a consequence of the production of organic acids. Chelation of $\text{Fe}^{2+/3+}$ and Ca^{2+} ions take place which checks them from fixing available phosphorus, secondly by depressing soil pH, which liberates mineral P-complexes, notably calcium (Walpola et al., 2012; Sible et al., 2021). An alternative way for increasing the balance of soil-available phosphorus would be hydrolysis of organic phosphorus in soil via formation of extracellular enzymes (Tarafdar et al., 2002). *Bacillus*, *Pseudomonas*, and *Rhizobium* bacteria are the furthestmost effective biostimulants for

phosphorus solubilization. (Sible et al., 2021). Certain biostimulants (for example biostimulants derived from *Azotobacter vinelandii*) may be utilised for detoxification of soil from hazardous contaminants (Ehaliotis et al., 1999; De Luca et al., 2020).

One of the most pronounced groups in the category of plant biostimulants is plant growth-promoting rhizobacteria (PGPR) that take possession of the plant rhizosphere (Du Jardin, 2015). There are certain beneficial groups of PGPR based biostimulants which include nitrogen-fixers i.e., *Rhizobium*, *Azotobacter* spp., *Azospirillum* spp., *Pseudomonas* spp. and *Bacillus* spp. (Basharat et al., 2021). Nitrogen (ammonia) obtained from atmospheric nitrogen (N₂) through nitrogen fixation by PGPR may be utilised by plants for enhancing their productivity (Bucaco, 2019). Enhancement of soil fertility and production can be done by increasing the accessibility of crop to essential nutrient elements this might be achieved through appliance of biostimulants that embraces PGPR, which have potential of releasing organic acids to solubilize insoluble phosphate and making it available (Basu et al., 2021). The accessibility of plants to the non-labile phosphorus reserve may be smoothen by phosphorus solubilising bacteria (PSB) by deliverance of recalcitrant form of phosphorus and making it more accessible to crops through organic acids and/or hydrochloric ions discharge. In same manner, certain phosphorus solubilising bacteria (PSB) -manufactured phytase are capable of liberating reactive phosphates from organic compounds (Bechtaoui et al., 2020). PGPR provide a great advantage in terms of making solubilised form of potassium available to plants as it can solubilize insoluble potassium through discharging of inorganic acids and making it available to crop plants, thus refining the agricultural productivity and health of crops while in case of inorganic fertilizer applied potassium, the solubilized form of rhizospheric potassium is not readily available for the reason that it has a tendency to form insoluble complexes (Sindhu et al., 2017). PGPR such as *Bacillus edaphicus*, *Acidithiobacillus* sp., *Ferrooxidans* sp., *Pseudomonas* sp., *Bacillus mucilaginosus*, *Burkholderia* sp., and *Paenibacillus* sp. have been acknowledged for releasing of potassium in its available form from potassium-bearing minerals in soils (Liu et al., 2012). Many strains of bacteria amend Fe (iron) availability by producing siderophores or organic acids. AgriLife (India) industrialized and developed the commercial formulation of *Acidithiobacillus ferrooxidans* which solubilizes Fe through liberation of organic acids (Wei et al., 2018).

4.6 Enzymes as Biostimulants

Enzymes are plant-descended hydrolysate biostimulants (Sestili et al., 2018). Polished enzymes may be employed as a commercial product in crop fields in form of biostimulants. Plants and microorganisms produce extracellular enzymes in soil and a range of biochemical practises in the soil can be dependent on organic N or P molecules and these enzymes function as biological catalysts to accelerate biochemical reactions (Nielsen et al., 2007). The Carbon cycle enzymes, in addition to phosphatases, are of significance for the reason that they may hasten residue breakdown and in high organic matter-based agroecosystems such as minimum tillage or cover cropping may provide a probable tool for its finest management. The polymer degrading enzymes comprise of cellulase and hemicellulase. These loftier polymers are degraded into monomers, if not possible then to miniature polymers by degrading enzymes and finally condenses them into more hydrolysable form. Mineralization of supplementary nutrients for forthcoming crop uptake is accelerated by degradation which creates a chain reaction (Sible et al., 2021). Numerous enzymes that trail an evident degradation route might be united in a precise combination for releasing specific organic components and nutrients. The biostimulant application influences the activity of catalase, dehydrogenase, and phosphatase in soil (Yousfi et al., 2021). In the biostimulants (PA-HE, RB, CGHE, WCDS) amended soils, soil dehydrogenase activity appreciably increased. With respect to the hydrolase enzymes, urease activity was also strongly stimulated by the addition of biostimulants to the soil (Tejada et al., 2011).

4.7 Biochar

A procedure identified as pyrolysis, involves the thermochemical breakdown of a fuel source without oxygen, produces biochar (Weber, K. et al., 2018; Leng, L. et al., 2018). Stable biochar is itself a decidedly carbonaceous compound that is resilient to degradation, its process and application to agronomic fields also functions as a source of C sequestration and, thus, it has been contemplated a “win-win-win” for agricultural use (Laird et al., 2008; Sible et al., 2021). The application of Biochar can appreciably upsurge the fertility and health of the soil through Carbon building i.e carbon sequestration which has been located to upsurge the yield by over and above 10% by augmenting various biochemical properties of soil (Jeffery et al., 2011; Sible et al., 2021).

5. Conclusion

As a first step, biostimulants are different from other agricultural inputs because they are more flexible in terms of achieving the desired response and are highly sustainable. For example, the application of seaweed extract as a bio-stimulant during planting may impact the microbial populations in the zone where the extract is applied, while in case of foliar applications at vegetative development stages are mainly intended to trigger certain signalling pathways for the reduction of abiotic stress. These biostimulants can be obtained from animal source or plant biomass. Elucidation of science behind the stimulation mechanism of biostimulants is still one of the unexplored parts. Also, the synergistic or antagonistic effect of biostimulants in accordance with synthetic chemicals is still unknown. Since single-season yield outcomes vary widely, farmers are wary, and successful biostimulant application on grower fields now needs a prescription approach which further will be surged up by many seasons of fine-tuning for a new technique to be successfully integrated.

7. References

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