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Biostimulants: Concept, Types and Way to Enhance Soil Health

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ABSTRACT

Soil has immense capacity to function as a vital living ecosystem that not only support plants and animals but also acknowledges survival of people, as a result there has been renewed interest in soil health but due to the environmental repercussions of poor management, such as soil erosion and nutrient contamination, the health of soil is deteriorating with an increasing rate. Application of biostimulants can prove to be a prominent tool for enhancing soil health. Biostimulants are compounds, microorganisms or other materials that are 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. Biostimulants enhance soil health (physical, chemical, biological properties) by targeting certain major prospects such as enhanced buffering capacity of soil, enhanced stability of aggregates and specific surface area. 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,

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Nitrogen-Fixing Bacteria and Phosphorus Solubilising Bacteria, Plant Growth Promoting Rhizobacteria briefly comes under the umbrella of biostimulants. These biostimulants can be extracted from various methods such as alkali hydrolysis, partial hydrolysis, pressurised liquid extraction,etc. Even while a biostimulants 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: Enzymes; plant biostimulants; sea weed extract; soil biota and soil health.

ABBREVIATIONS

SWE	:	*Sea Weed Extract*
T-RFLP	:	*Terminal Restriction Fragment Length Polymorphism*
TPF	:	*Triphenyl Formazan*
CNFB	:	*Cellulolytic Nitrogen Fixing Bacteria*
OC	:	*Organic carbon*
HA	:	*Humic Acid*
PGPR	:	*Plant Growth Promoting Rhizobacteria*
PSB	:	*Plant Solubilizing Bacteria*
NBRIP	:	*National Botanical Research Institute's Phosphate Growth Medium*
PPK	:	*Polyphosphate Kinase Gene*
PQQ	:	*Pyrroloquinoline Quinone*
VAM	:	*Vascular Arbuscular Mycorrhiza*
AMF	:	*Arbuscular Mycorrhizal Fungi*
CAGR	:	*Compound Annual Growth Rate*
PHs	:	*Protein Hydrolysates*
PBs	:	*Plant Biostimulants*
WCDS	:	*Wheat Condensed Distillers Solubles Enzymatic Hydrolizate*
PA-HE	:	*Hydrolyzed Poultry Feathers*
CGHE	:	*Carob Germ Enzymatic Extract*
RB	:	*Rice Bran Extract*

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 [1]. 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 [2]. Developing innovative technologies and tactics to increase the partial factor productivity and resource use efficiency especially of water and fertilizers as well as to augment productivity of field crops both under optimal and suboptimal conditions which is at most critical to safeguard food security keeping in mind to focus conserving quality and health of soil in accordance with providing ample opportunities for farmers [3]. 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 [4] and may cause various environmental issues and even influence human health. Also, the industrial manufacturing of chemical fertilizers influences the energetics of the system as it manufacturing is a highly energy-intensive procedure [5] that has been linked to hefty boost in global CO_2 emissions [6]. Organic fertilizers, such as compost, sludge, or manure, offer the assistance of utilizing nutrients that are before now present in the Agro-ecosystem and require 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 of nutrients is not in synchronized form and therefore is subjected to various losses [7,8]. One way to offset this shortcoming is to cultivate such crops which have a more robust root system 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 biostimulants to crop leaves, seeds, or soil to fuel up root development [4]. 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 [9]. A diamond cut technology would be the application of natural plant biostimulants (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 [7]. 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 [10]. Other substances, chiefly synthetic and not extracted from organic biological resources, may have stimulating properties but are not so far considered as PBs [10,11]. PBS 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 [12]. PBs industry is proliferating at a rapid rate with annual growth rate of 12%.The results obtained from PBs application to soil and plants depends on variety of factors like method of application(either foliar applied or soil applied), time and duration of application, therefore the understanding of relationship between PBs and crop physiology is must [13]. Despite of immense importance, basic categorization and understanding about biostimulants is still lacking. Even after recent advancements in PBs targeting molecular phyiology of crop plant the categorization of PBs is not rigid. The endeavour of this review article is to build up a basic, indispensable concept and understanding about biostimulants especially PBs, their major cataloguing and highlighting the after math of biostimulants 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 humans, animals, and plants [14,15]. Herve, presented the primary factual conceptual approach to biostimulants, he proposed that the development of "bio-rational products" (later now known as biostimulants) 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 [16,15]. The first systematic dialogue on biostimulants was established by Yakhin and hence he is known for building conceptual groundwork for the current biostimulants science [15,17]. It has been proved by several researchers that application of biostimulants to field crops can lift crop yields, nutritional content and the capital expended can be reduced, the result of which is that they are frequently being promoted to be used in agricultural management strategies targeting at curtailing chemical inputs, improving productivity, and restoring natural agro-ecosystem balance [18].

The new Regulations by (EU) 2019/1009, delineates that plant biostimulants as follows: “Substances and microorganism which can stimulate natural processes to improve nutrient uptake, nutrient efficiency and crop quality”. Some belief 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 [19]. PBs 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* [20].

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[18].

The Compound Annual Growth Rate (CAGR) of biostimulants was 10.65% till 2019 which proves biostimulants especially PBs 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 [21]. Since not all of the cited parts or components are "biological" in origin hence the word "bio" used in biostimulants is rather confusing. 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 [18].

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 of classification [17]. Configuration of biostimulants 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 [22]. 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 [23]. The following sorting has been extensively acknowledged as;

3.1 Plant Biostimulants of Microbial Origin

These Microbial derived PBs may be progressed with fungi, bacteria, and AMF (Arbuscular Mycorrhizal Fungi) [9]. 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 [18]. PBs of microbial origin or simply microbial inoculants group predominantly include Plant Growth Promoting Rhizobacteria (PGPR) and endophytic fungi such as AMF (Arbuscular Mycorrhizal Fungi) and *Trichoderma spp* [25, 7]. 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 [25].

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 [26]. 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 [27,28]. 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 [26,29]. 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 [30]. PBs 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 [28].

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 biostimulants [31]. 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 Sea Weed Extracts (SWEs) is Alkali Extraction (sodium or potassium solutions), with or without heating [32,33]. 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 [33]. The most commonly utilized seaweed species for extraction of SWEs include *A. nodosum*, *Macrocystis pyrifera*, *Ecklonia maxima*, *Ecklonia radiata*, *Durvillaeapotatorum*, *Durvillaea antarctica*, *Laminaria digitata*, *Laminaria* species, *Sargassum* species and *Fucus serratus* [23,33,34].

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 [23]. 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 [27] 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 [35]. 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 [35, 23, 4]. 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) [23]. The treated species with protein hydrolysates showed boosted carbon and nitrogen metabolism, enhanced nutrient availability and uptake by the crop, and ultimately upgraded the nutrient use efficiency [36].

3.5 Humic Substances Derived Plant Biostimulants

PBs can be obtained from OC (organic carbon) centered products such as manures, compost, etc. by hydrolysis reaction [37]. The beneficial effect of humic acid (HA) 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 [38]. 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 (HS) constitute of recalcitrant chemicals which are resistant to further breakdown. These recalcitrant molecules are commonly classified as humic substances [39]. Understanding of average molecular mass and mass distribution (determined using Ultracentrifugation, Sedimentation Velocity, Equilibrium Sedimentation and Archibald approach) is important characterisation of HS [40]. HS can be extracted from various sources (manures, compost, soil, water etc) by several methodologies like alkali hydrolysis, acid hydrolysis.

3.6 Biochar as Biostimulant

A procedure identified as pyrolysis and gasification, involves the thermochemical breakdown of a fuel source without oxygen, produces biochar [41,42]. 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 [12,43]. Application of Biochar to soil has the potential to influence soil fertility parameters such as quantity: intensity ratio, buffering capacity of soil, water and nutrient retention capacity [44-46]. The production technology and conditions involved in biochar largely influences all its properties. Also, the selection of feedstock (for example lignocellulosic source of biochar is better compared to non-cellulosic biomass), high temperature treatment (HTT) is important in deciding the quality of biochar [47].

Seaweed Extracts, Humic and Fulvic Acids, Nitrogen Fixing Bacteria, Phosphorus Solubilizing Microorganisms (PSM), AMF, PGPR, Enzymes, and Biochar fall under the major categorization of biostimulants [23, 12, 48].

4. IMPACT OF BIOSTIMULANTS ON SOIL HEALTH

PBs enhance soil health (physical, chemical, biological properties) by targeting certain major prospects such as enhanced buffering capacity of soil which influences all the major biochemical process occurring in soil, enhances stability of aggregates by increasing, increases specific surface area.Fig.1 depicts major mechanisms by which PBs improves soil health.

Application of PBs to soil improves its CEC (cation exchange capacity) which influences the buffering capacity of soil which ultimately either directly or indirectly influences all the major mechanism occurring in soil. Availability of nutrients to plants is enhanced by PBs by mineralising organic form of nutrients to inorganic form and making it easily available to plant which has been described in Table 1.

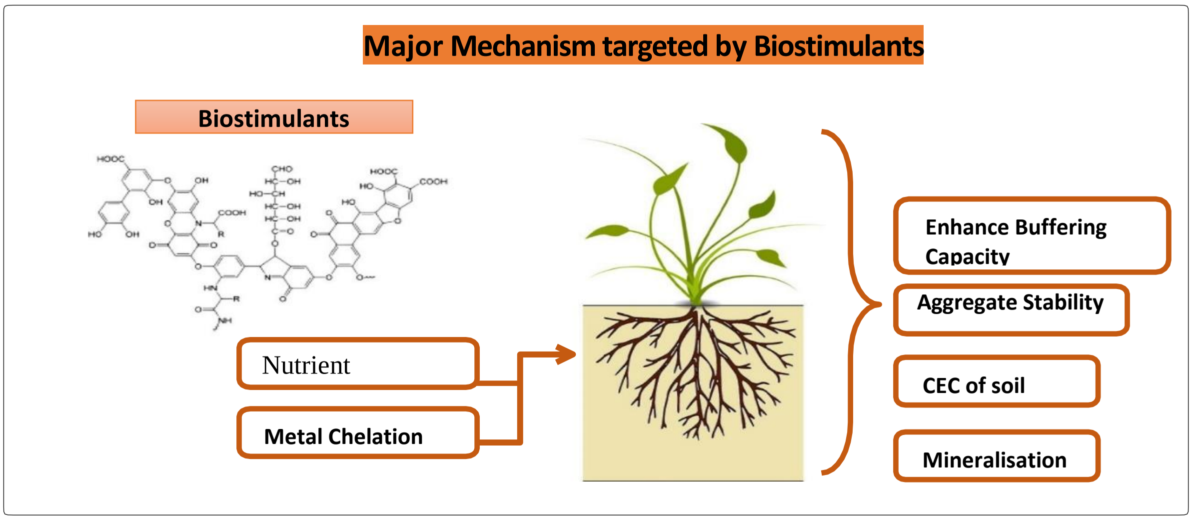

Fig 1. Major mechanism followed/targeted by biostimulants

Table 1. Different methods deployed for extraction of PBs and their influence on soil health of varied soil types

Briefing of Effect of Biostimulants application to soil				
Biostimulant	Extraction /Isolation method used	Soil characteristic	Effect on soil	source
Humic acid	Leaf compost with 0.5 1 NaOH solution (1:5) through 24-hour agitation and precipitation with $6NH_2SO_4$ up to pH 2	Clay loam	Addition of HA 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 and Mindari, [49]
Commercially formulated HA	Not specified	pH :7.84, clay loam	Amend plant nutrition by intensifying the uptake of N, P, Mg, and Ca thus affecting	Nasiroleslami, et al. [50]

Briefing of Effect of Biostimulants application to soil				
			grain yield. In addition, HA enhances Fe and Zn uptake.	
SWE- *Durvillaea potatorum* and, Ascophyllum *nodosum* 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. [51]
SWE - *Ascophyllum nodosum* 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, *Phingomonadaceae*, *Rhizobiaceae* and *Pseudomonadaceae* .	Renaut et al. [52]
SWE *Lessonia flavicans* based	Pressurized Liquid Extraction	sandy loam	The activity of urease, proteinase, and phosphatase compounded. based on *Lessonia nigrescens* and *Lessonia flavicans*.T-RFLP (Terminal Restriction Fragment Length Polymorphism) analysis showed increased abundance of fungal community.	Wang et al. [53]
SWE *Macrocystis pyrifera* based	Soft Extraction (traditional method)	haplic luvisol	Augmented hydrogenase activity (mg Triphenyl Formazan /10 g) in soil by 32% .	Onet et al. [54]
Inoculation with CNFB (Cellulolytic Nitrogen Fixing Bacteria)	Isolated on a nitrogen free (NF) - CMC/Cellulose agar medium	Not Specified	Augmented the physicochemical, biological properties of soil and also increased the SOC (Soil Organic Carbon)	Harindintwali et al. [55]
Inoculation with PSB	National Botanical Research Institute's Phosphate Growth Medium for Isolation and	Shandong brown soils	Compounded richness of bacterial community at both phylum and genus level, Proteobacteria	Wan et al. [56]

Briefing of Effect of Biostimulants application to soil				
	acclimation		dominated at genus level while *Cellvibrio* at species level. Transformation of insoluble tricalcium phosphate into soluble phosphorus takes place and this is manifested by polyphosphate kinase gene (*ppk*) and pyrroloquinoline quinone (*pqq*)	
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 et al. [57]
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 et al. [58]
Inoculation with *Bacillus myloliquefaciens* (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. [59]

Table 2. Chemical composition of Humic and fulvic acids [12]

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

4.1 Humic Acid

Understanding the fundamental system, how plant responses to the biostimulants application, is a significant bedrock for the practical applicability of humic substances (HS) in the fields. For successful application the foremost step definitely will be an enhanced understanding of the consequence of HS application on various biological cycles such as carbon and nitrogen cycles which are directly correlated to primary metabolism of plant [61,61].

The enhanced carbon (C) content of these organic molecules may serve up as an undeviating energy source for a variety of soil biota., consequently encouraging their activity and potentially ensuring more productive soil [12]. Humic and fulvic acid appliance has proven its worth in enhancement of soil's structure via amplified stability of aggregates [12]. HA, 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 which ensures comparatively superior yield when applied before sowing as pre-plant soil amendment [62]. HA 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 [63]. The release of protons and exudates, which is guided by plant roots have illustrated multifaceted dynamics of association or dissociation of these HS supra-molecular colloids. As HS 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 HA. 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 [64]. 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 [66-67]. 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 HA application [66].

4.2 Sea Weed Extract

The application of sea weed grounded biostimulants 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 [48,52]. Further, the SWE (seaweed extract) comprise a wide range of minerals and chemicals that operates as chelating agents and assist soil health benefits [42,68]. Application of SWE adds to soil gel formation, water retention, and soil aeration through their polysaccharides, which are found in soil [69]. In supplementing to improve soil structure and texture, application of extract to soil also aims at enhancing porosity, aeration and moisture retention capacity of soil [70]. 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 [71] 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 [23]. 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 [72]. 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 [12,32].

When *Lessonia nigrescens* and *Lessonia flavicans* based SWE 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 (Terminal Restriction Fragment Length Polymorphism) analysis which exposed that the soil fungal community has been considerably transformed after seaweed extract application to soil [57,73].

4.3 Arbuscular Mycorrhizal Fungi

Majority of plant species (about 90%) have symbiotic relationships with a mycorrhizal fungus [74]. 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 [75]. 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 [76]. As an outcome of Arbuscular Mycorrhizal Fungi (AMF), nutrient levels are more stabilised, and the soil system is more proficient. Biological and abiotic components in soil are interdependent and AMF hyphae form a connection between the two components through the plant's roots inside the pedosphere [77]. 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 PBs, 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 [26,78]. 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+} [24].

4.4 Nitrogen Fixing Bacteria, Phosphorus Solubilizing Bacteria and Plant Growth Promoting Rhizobacteria

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) [12,79]. 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 [80]. 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 crumby structure of the soil [81].

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 [82]. 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 $Fe^{2+/3+}$ 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 [12,83]. An alternative way for increasing the balance of soil-available phosphorus would be hydrolysis of organic phosphorus in soil via formation of extracellular enzymes [84]. *Bacillus, Pseudomonas,* and *Rhizobium* bacteria are the furthermost effective biostimulants for phosphorus solubilisation [12]. Certain biostimulants (for example biostimulants derived from *Azotobacter vinelandii*) may be utilised for detoxification of soil from hazardous contaminants [85,86].

One of the most pronounced groups in the category of PBs is plant growth-promoting rhizobacteria (PGPR) that take possession of the plant rhizosphere [23]. 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. [87]. Nitrogen (ammonia) obtained from atmospheric nitrogen (N2) through nitrogen fixation by PGPR may be utilised by plants for enhancing their productivity [88]. 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 [89]. 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 [90]. 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 [91]. PGPR such as B*acillus edaphicus, Acidothiobacillus sp., Ferrooxidans sp., Pseudomonas sp., Bacillus mucilaginous, Burkholderia sp.,* and *Paenibacillus sp*. have been acknowledged for releasing of potassium in its available form from potassium-bearing minerals in soils [92] 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).

4.5 Enzymes as Biostimulants

Enzymes are plant-descended hydrolysate biostimulants [93]. 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 practices in the soil can be dependent on organic N or P molecules and these enzymes function as biological catalysts to accelerate biochemical reactions [25]. 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 [23]. Numerous enzymes that trail an evident degradation route might be united in a precise combination for releasing specific organic components and nutrients. The biostimulants application influences the activity of catalase, dehydrogenase, and phosphatase in soil [94] In the biostimulants (Hydrolysed Poultry Feathers, Rice Bran Extract, Carob Germ Enzymatic Extract, Wheat Condensed Distillers Solubles Enzymatic Hydrolizate) 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 [37].

4.6 Biochar

Biochar is considered to be of high quality if it has CEC value of more than 20 cmolc kg^{-1} and specific surface area of more than 10 m^2 g^{-1} Biochar descended from crop residue is typically low in major nutrients especially N, for instance biochar derived from wood waste was found to contain less than 0.3% N, while biochar derived from manure (cow manure, poultry litter, pig manure) contained about 4.2 % N [95,96]. Presence of biochar is highly correlated to the soil biota which improves carbon stability, heavy metal transformation and enhances nutrient recycling from organic to inorganic form [97]. It has been demonstrated by several researchers that application of biochar to soil improves its hydraulic conductivity, water holding capacity and reduces its compaction and bulk density [98, 96]. It is worth to mention that after applied to the field, biochar gradually increases its CEC (cation exchange capacity) and SSA (specific surface area) and this increase in CEC and SSA is the prominent reason for improving soil health. Also, SSA, O/C ratio and H/C ratio are important parameters for characterising the quality of biochar [96] Biochar enriches 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 [99,12]. Biochar improves the total pore volume, increases pH of soil [100, 101] which in turn increases the availability of nutrients under acidic condition by raising the pH of soil. The increase in pH is because of basic feedstock and binding of H^+ [101-103]. Biochar improves the pore distribution between macropores (>200nm), micropores (<2 nm) and mesopores (2-50 nm) and with higher surface charge is responsible for better retention of nutrients, water and encourages abundance and activity of soil biota [104-106].

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 biostimulants 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 signaling 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 biostimulants application on grower fields now needs a prescription approach which further will surged up by many seasons of fine-tuning for a new technique to be successfully integrated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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