Application of Plant Biostimulants as New Approach to Improve the Biological Responses of Medicinal Plants- A Critical Review

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Abstract

The aim of this review was to represent a category of applied plant biostimulants and to highlight the effect of their application on morphological and phytochemical properties of medicinal plants in *in vivo* and *in vitro* conditions and their mechanism of action. Plant biostimulants, safe for both human beings and the environment, are substances or materials, except for nutrients and pesticides, which have the ability to beneficially modify plant growth and have been accepted over the past decade. Plant biostimulants have natural and synthetic origin. The natural biostimulants contain amino acids, bacteria, seaweed, yeast, chitosan, phytohormones, and also plant growth regulators (synthetic hormones), phenolic compounds, inorganic salts, essential elements, and other different substances are some examples of synthetic biostimulants. The plant biostimulants through different mechanisms of action (such as nitrogen assimilation) are sustainable management practice for production of medicinal plants, increasing biomass production, and enhancing secondary metabolites synthesis and it is investigated and shown in many studies in recent years. Furthermore, there are many reports on positive effects of biostimulants and elicitors application on medicinal plants in *in vitro* culture condition. Some products of companies active in the formulation of biostimulants in Iran are mentioned in this review. In conclusion, yield response in medicinal plants to biostimulants application showed that each medicinal plant responds in a different way based on the chemical composition and components of biostimulants, the timing and rate of application.

Keywords: Biostimulants, Elicitors, Medicinal Plants, Plant growth regulators, Secondary metabolites

Introduction

A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content [1]. The European Union (EU) regulations force restriction on the utilization and production of pesticides; thus, this needs searching for new and effective substances which are environment friendly. One of the solutions is to use plant biostimulants, which are safe both for human beings and for the environment and in particular, valuable for reducing chemicals in agriculture. In recent years, plant biostimulants are being extensively used in farming and cultivation. Positive effects of its application have yielded extraordinary results as confirmed by many studies [2-8]. Meanwhile, for a long time now, plant biostimulants have been thought to be biological (that is, natural) substances that only stimulate processes within plants [9]. A challenge is now to use this knowledge and these tools for the characterization of biostimulants and their effects on a wide range of cultivated plants [1]. The application of plant biostimulants, characterized here as a substance or material, except for nutrients and pesticides which has the ability to beneficially modify plant growth has been accepted over the past decade and it is anticipated that the market for plant biostimulants will surpass US$2 billion by the year 2018 and a formulation annual growth rate of 12.5 % from 2013 to 2018 [10, 11]. The largest market for plant biostimulants in 2012 was Europe. The European biostimulants industry council (EBIC) reported that in 2012 over 6.2 million hectares were treated with plant biostimulants in Europe (characterized as the European economic area). Some of biostimulant manufacturers put a lot of emphasis on what their products could do and reported common benefits of biostimulants (Table 1). Challenges for the development of biostimulants are of scientific, technical and regulatory nature. The main scientific challenge is the complexity of the physiologyical effects of biostimulants. Technical challenges include the formulation and blending of biostimulants with other fertilizing materials and/or plant protection products. Regulatory challenges are related to the categorization and premarket assessment of biostimulants, and to intellectual property [1].

Plant biostimulants are organic materials that appear to impact several metabolic procedures such as respiration, photosynthesis, nucleic acid synthesis and ion uptake and when applied in small quantities, improve the plant growth and development [12] or in other words, a mixture of two or more PGRs or combination of these with other substances (amino acids, nutrients, vitamins) is known as a plant growth promoter or plant biostimulant. Plant biostimulants are effective when applied in small doses, thus leads to the plant growth, and production enhancement [13]. In general, they stimulate metabolic processes for more yields in plants [9]. In addition, the application of plant biostimulants in condition of environmental stress can diminish effects of stress and improve soil water holding capacity, root growth and yield. Specifically, they decrease the application of mineral fertilizers by increasing the amount of micro- and macro-nutrients taken up by plants, positively affecting root morphology and plant growth [14-18]. They also show hormone-like activity and impact plant metabolism by interacting with biochemical processes and physiological

Table 1- Common benefits of biostimulants reported by manufacturers [159]

▪ Stimulate plant responses and work in all weather conditions	▪ Stimulate plants' immune system
▪ Increase profits, cut operating costs, lead to 50% reduction in fertilizer	▪ Produce better color
▪ Increase natural plant toxins, repelling pests	▪ Result in better performance
▪ Increase microbial root protection from soil pathogens	▪ Produce deeper roots
▪ Increase soil nutrient reserve up to 3000%	▪ Improve stress tolerance
▪ Improve root developement	▪ Accelerate establishment
▪ Build yields	▪ Increases cation exchange
▪ Improve taste and shelf life	▪ Enhances fertilization and reduces leaching
▪ Improve drought tolerance	▪ Detoxify chemical residues and heavy metals
▪ Increase nutrient uptake	▪ Make urea a long life nitrogen
▪ Improve seed germination rates	▪ Increase stomata opening and plant transpiration

mechanisms, such as glycolysis and nitrogen assimilation [19, 20, 21]. Recent consideration has been given to decrease contamination sources in present agriculture. One of the procedures to reduce soil pollution is the application of biostimulants, which has turned out to be as a safety nature of plant growth regulators, polyamines and vitamins [22].

Application of different plant biostimulants in various concentrations and diverse mechanisms of action has resulted different effects on medicinal plants. This research is a selection from the most important applications of these products on aromatic and medicinal plants. Plant biostimulant products are novel in agronomy. However they are more efficient in medicinal plants due to possibility of genetic manipulation in synthesis pathways of secondary metabolites. The aim of this review was to represent a category of applied plant biostimulants and to highlight the effect of their application on morphological and phytochemical properties of medicinal plants under *in vivo* and *in vitro* conditions and their mechanism of action.

Category of plant biostimulants

In spite of the fact that the expression "biostimulant" has been used for a long time, it is not completely defined, yet. According to the European biostimulants industry council (EBIC) [23], biostimulants are not: ***a*)** Fertilizers, regardless of the presence of nutrients in the products. ***b*)** Crop production products because they act only on the plants vigor and do not have any direct action against pests and diseases. ***c*)** Crop nutrition and crop protection, but as complementary to these products. Actually, the term biostimulantsis related to chemicals from different sources, biotic or abiotic, as well as physical factors, that can trigger a response in living organisms resulting in accumulation of secondary metabolites. Then, biostimulants and elicitors are beneficial factors for enhancement of plant valuable compounds [24-27].

The biotic elicitors have biological origin, derived from the pathogen or from the plant itself (sometimes called endogenous biostimulants). Biotic compounds can be of defined composition, when their molecular structures are known, or have a complex composition when they comprise several different molecular classes making difficult to define a unique chemical identity [28] (Table 2). On the other hand, abiotic elicitors do not have a biological origin and are classified in physical

Table 2- Biotic biostimulants or elicitors and production of secondary metabolites [160]

SI. No.	Plant species	Product	Biotic Elicitor
1	*Arabidopsis*	Camalexin, indole glucosinolates	*Erwinia carotovora*
2	*Bidens pilosa*	Phenylheptaryn	Fungal culture filtrate
3	*Brugmansia candida*	Hyosujamine	Hemicellulase
4	*Capsicum annuum*	Capsidol	Cellulase
5	*Catharanthus roseus*	Indole alkaloids	Fungal elicitor
6	*Catharanthus roseus*	N-acetyl-tryptamine	*Pythium aphanidermatum*
7	*Catharanthus roseus*	Ajmalicine	*Trichoderma viride*
8	*Carthamus tinctorious*	Polyacetylenes	Fungal polysaccharide
9	*Cicer arientium*	Medicarpin, Maackiain	*Ascochyta rabiei*
10	*Cupressus lusitanica*	Beta-thujaplicin	Fungal elicitor
11	*Datura starmonium*	Lubimin	Fungal spores
12	*Dioscorea deltoidea*	Steroid (Diosgenin)	Fungal mycelia
13	*Glycine max*	Glyceollin	Fungal glucan
14	*L. erythrorhizon*	Shikonin	Agaropectin
15	*Medicago sativa*	Isoflavonoid	*C. indemuthianum*
16	*Medicago sativa*	Phytoalexins	Fungal elicitor
17	*Medicago truncatula*	Beta-amyrin	Yeast elicitor
18	*Papaver somniferum*	Morphine, codeine	*Vertcillium dahliae*
19	*Petroselinum crispum*	Enzymes	Fungal Elicitor
20	*Phaseolus vulgaris*	Krevitone	Fungal polysaccharide
21	*Ruta graveolens*	Rutacridone epoxide	Chitosan
22	*Salvia miltiorrhiza*	Diterpenoid tanshinones	Yeast elicitor
23	*Silybum marianum*	Silymarin,	Yeast extract
24	*Various plant cells*	Enzymes and sec. metabolites	*Erwinia carotovora*

factors and chemical compounds (Table 3). The classification described above only notes to the nature of the biostimulants and elicitor. But these compounds may be also classified, according to the interaction plant biostimulants, into two groups: ‘general biostimulants’ which are able to trigger defense responses both in host and non-host plants and ‘race specific biostimulants’ which induce responses leading to disease resistance only in specific host cultivars, depending on the simultaneous presence of virulence and resistance genes in the pathogen and plant, respectively [29]. It is now clear that there are several different classes of components that can completely substitute for fungal biostimulants in the elicitation effect. These include poly or oligosaccharides such as chitin, and chitosan and their fragments (e.g., chitooligosacharides), xyloglucans, laminarin and other h-glucans and their fragments and oligogalacturonides, proteins (e.g., harpin or elicitins such as cryptogein) or peptides (e.g., 13-pep, systemin, and flg22), as well as lipid derivatives such as syringolide, nod factors and lipopolysaccharides. Poly-oroligosaccharides are the most well studied signal molecules in elicitor signal transduction [30].

These plant biostimulants of natural or synthetic origin comprise of various organic and inorganic components. Ingredients from 15 applied biostimulants are mentioned in Tables 4 and 5 [32]. However it is possible to categorize these products based on their components:

Table 3- Abiotic biostmulants or elicitors and production of secondary metabolites [160]

SI. No.	Plant species	Abiotic Elicitor	Product
1	*Arabidopsis*	Oxidative stress, amino acid starvation	Camalexin
2	*Atropa belladona*	Cu^{2+}, Cd^{2+}	Tropane alkaloids
3	*Catharanthus roseus*	Diethyl amino ethyl dichloro phenyl ether	Indole alkaloids
4	*Catharanthus roseus*	Vanadium sulphate	Catharanthine
5	*Capsicum annuum*	Arachidonic acid	Capsidiol, Rishitin
6	*Capsicum frutescens*	Curdlan, Xanthan	Capsaicin
7	*Coleus blumei*	Methyl jasmonate (MeJA)	Rosmarinic acid
8	*Coleus forskolin*	Methyl jasmonate (MeJA)	Forskolin
9	*Cupressus lusitanica*	Methyl jasmonate (MeJA)	β-thujaplicin
10	*Datura stramonium*	Metal ions: Al^{3+}, Cr^{3+}, Co^{2+}, Ni^{2+},Cu^{2+}, Zn^{2+}	Sesquiterpenoids
11	*Daucus carota*	Salicylic acid	Chitinase
12	*Glycyrrhiza echinata*	Na-alginate	Echinatin
13	*Hyoscyamus albus*	Copper sulphate	Phytoalexin
14	*Hyoscyamus albus*	Methyl jasmonate (MeJA)	Phytoalexins
15	*L. erythrorhizon*	Activated carbon	Benzoquinone
16	*Medicago truncatula*	Methyl jasmonate (MeJA), UV light	Triterpene β-amyrin
17	*Panax ginseng*	Low-energy ultrasound	Saponins
18	*Rauvolfia canescens*	Jasmonic acid	Secondary metabolites
19	*Silybum marianum*	Methyl jasmonate (MeJA)	Silymarin
20	*T. canadensis, T. cuspidata*	Methyl jasmonate (MeJA)	Taxoids
21	*T. wallichiana*	Methyl jasmonate (MeJA)	Taxanes
22	*Taxus chinensis*	Trifluoroethyl salicylate (TFESA)	Taxuyunnanine C (Tc)
23	*Taxus spp.*	Arachidonic acid	Taxol
24	*Valeriana wallichii*	Colchicine	Valepotriates

Table 4- Ingredients from applied biostimulants [159]

Activated nutrients	Cytokinin	Metabolites	Polysaccharides
Active Humic acids	Disaccharides	Micronutrients	Proteins
Amids	Enzymes	Minerals	Scientifically balanced formulation (No ingredients mentioned)
Amino acids	Fermentation materials	Monosaccharides	Sea kelp
Antioxidants	Fungi	Mycorrhiza	Seaweed
Bacteria	Gibberllic acids	Natural wetting agents	Secondary nutrients
Carbohydrates	Growth simulators	N-fixing Bacteria	Simple sugars
Carbon-rich- organics	Humic substances	Non-ionic wetting agents	Soil conditioners
Cellulose fiber	Humic/Fulvic acids	Nutrient broth	Sugar acid chelates
Chelated micronutrients	Hydrated organic proteins	Organic chelates	Vitamins
Chelates	International metabolites	Peptides	Wetting agents
Chemical activators	Invert sugars	PGRs	Yeast
Complex sugars	Kelp extract	Plants extracts	Yucca extract wetting agent
Cultured living microorganisms	Lignin	Plant hormones	
Cyanobacteria	Manure extract	Plant nutrients	

Table 5 - A summary of six commercial biostimulant formulations showing major and minor active component, active compounds with targeted stress factors, supporting references and recommended field application range or rate [161]

Formulation name	Major component	Minor component	Active compounds	Target stress factors	Field application range or rate/ha	Mid-point of application rate*
Rygex	Humic acids, algal, extracts	Amino acids	Humic acid, amino acid, alginates and minerals	Drought and salt stress	Foliar: 20-30 kg in 300 l	25 kg/300 l
Algavyt	Algal extract	Amino acids	Mannitol, amino acid and minerals	Drought and cold stress	Foliar 100-150 g in 100 l	125 g in 100 l
Ryzoset	Plant extracts, poly saccharids	Lipids	Amino acid, lipids, saponins	Enhanced root growth to reduce abiotic and biotic stresses	Foliar: 20 l in 300 l	-
Manek	Fatty acid/vegetable oil	Plant extracts	Fatty acid, alkaloid, phenol, diterpenes, glucosinolates, tannins	Enhanced resistance to biotic stress	Fertigation: 15 l in 300 l	-
Ecoryg	Plant peptides, amino acids	Minerals	Amino acid, proteins and minerals	Drought stress	Foliar: 70-150 kg in 300 l	110 kg/300 l
Algavyt Zn/Mn	Algal extracts	Amino acids, minerals	Alginates, amino acid, minerals	Biotic stress	Foliar: 100-150 g in 100 l	125 g/100 l

*Mid-point of the recommended application range was used as test concentrations for the DLS analysis of Rygex, Algavy, Ecoryg and Algavyt Zn/Mn and recommended application rate for Ryzoset and Manek only

A) Natural derivatives are based on free amino acids, extracts from seaweed and fruit, effective microorganisms, humic substances, and chitosan [11].

A-1) Amino acids

Amino acids of glutamine, glutamate, aspartate, and asparagines serve as pools and transport forms of nitrogen, as well as in adjusting the carbon/nitrogen proportion. Other amino acids such as tryptophan, methionine, proline and arginine cause more resistance in plants against biotic and abiotic stress conditions either directly or indirectly by serving as precursors to secondary compounds and hormones [31]. Recently, studies have demonstrated that amino acids can directly or indirectly impact the physiological activities in plant growth and development. Likewise, amino acids are understood as bio-stimulants, which effectively improve effects the plant growth and yield and significantly relieve the injuries caused by abiotic stresses [23]. Amino acids with their widely use for the biosynthesis of a large variety of nonproteinic nitrogenous materials, i.e. pigments, vitamins, coenzymes, purine and pyrimidine bases are the most important biostimulants. Studies showedthe direct or indirect influence of amino acids on the physiological activities of plant [32, 54].

A-2) Free-living nitrogen fixing bacteria

Free-living nitrogen fixing bacteria, for example; *Azotobacter chroococcum* and *Azospirillum lipoferum,* were found to have the ability to fix nitrogen as well as the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption ofnutrients, and photosynthesis [32, 33]. They are blends of one or more things such as microorganisms, yeast, seaweed extracts, plant growth promoting rhizobacteria (PGPR) and humic acid trace elements, enzymes and plant hormones [30]. The active elements of plant biostimulantsare many organic compounds (phenols, vitamins, polysaccharides, betaines, etc.), growth regulators, algae, humus, extract from grapefruit, garlic and also macro and micro elements [34, 35, 36].

A-3) Seaweed

Seaweeds with about 9,000 species of macroalgae are classified into three main groups based on their pigmentation (for example, Phaeophyta, Rhodophyta, and Chlorophyta; or the brown, red, and green algae, respectively). Brown seaweeds with about 2,000 species are the most used type in agriculture [37] and, among them, *Ascophyllum nodosum* (L.) Le Jolis is the most researched [38] (Table 6). Seaweeds as sources of organic matter and fertilizer nutrients are used for soil conditioners in recent centuries [37, 39, 40]. They can overcome nutrient deficiency in crop plants and decreasing the use of expensive chemical fertilizers [41]. They are applied as biostimulants or biofertilizers to increase plant growth and yield. So many researches results have shown beneficial effects of seaweed extract application on plants, such as early seed germination, enhanced crop performance and yield, increasing resistance to biotic and abiotic stress, and improving postharvest shelf-life of degenerating products. Seaweed extracts effects are the same as liquid fertilizers and biostimulants since the existence of cytokinins, auxins, gibberellins, betaines, macronutrients (Ca, K, P) and micronutrients (Fe, Cu, Zn, B, Mn, Co, and Moin their components, necessary for the development and growth of plants[42-52].

Table 6- Some of commercial seaweed products used to production of medicinal plants

Product	Manufacture	Seaweed
Stimplex and Acadian	Acadian SeaPlant, Canada	*Ascophyllum nodosum*
Thorvin	Thorvin Inc., Norway	*Ascophyllum nodosum*
Algifert	Algea	*Ascophyllum nodosum*
Nitrozyme	Agri-Growth International Inc., USA	*Ascophyllum nodosum*
SeaCrop	Atlantic Laboratories Inc., USA	*Ascophyllum nodosum*
Kelpro	Tecniprocesos Biologicos, USA	*Ascophyllum nodosum*
HighTide	Green Air Products Inc., USA	*Ascophyllum nodosum*
Aquamax	Aqua Maxx Inc., USA	*Ascophyllum nodosum*
Guarantee	Main Stream Organics, USA	*Ascophyllum nodosum*
BioZyme	BioZyme, Inc, St. Joseph, Missouri, USA	*Ascophyllum nodosum*
Gofar	Gofar Agro Specialries, China	*Ascophyllum nodosum*
Algagreen	Oilean Glas Teao, Ireland	*Ascophyllum nodosum*
Alga Complex	BioAtlantis Ltd., Ireland	*Ascophyllum nodosum* seaweed with added N, P, K, boron, manganese zinc and copper
Ecolicitor	Bioatlantis Ltd, India	*Ascophyllum nodosum*
Biovita	PI industries Ltd, India	*Ascophyllum nodosum*
Plantin	Plantin SARL, France	*Ascophyllum nodosum*
Algifol	Neomed Pharma GmbH, Germany	*Ascophyllum nodosum*
Liquid Kelp	SeaGold, Australia	*Ascophyllum nodosum* and *Passadonius australus*
Kelprosoil	Productos del Pacifico, Mexico	*Ascophyllum nodosum*
Goemar	Goemar Laboratories, France	*Ascophyllum nodosum*
Seasol	Seasol International, Australia	*Durvillaea potatorum*
Seaweed	Natrakelp, Australia	*Durvillaea potatorum*
Profert	BASF, Chile	*Durvillaea antartica*
Rygex	Agriges, Italy	*Ascophyllum nodosum* and *Laminaria*
Ocean	VNET, India	*Ascophyllum nodosum* and *Laminaria*
Agrokelp	Aldas Bioderivados Marinos, Mexico	*Macrocystis pyrifera*

A-4) Yeast

Yeast extract is known as a natural source of thiamine, riboflavin, niacin, pyridoxine and vitamins B_1, B_2, B_3, and B_{12}, cytokinins and many of the nutrient elements as well as organic compounds i.e., protein, carbohydrates, nucleic acid and lipids that stimulates cell division and enlargement. [53-56]. Foliar application of yeast extract and ascorbic acid increased vegetative growth of eggplant [57]. These organisms also facilitate the growth of plants by improving the uptake of nutrients and production of some phytohormones and convert insoluble form of phosphorous into soluble one enhancing phosphorous availability to plants [58].

A-5) Chitosan

Chitosan is formed from chitin, a co-polymer of *N*-acetyl-*d*-glucosamine and d-glucosamine, when over 80% of the acetyl groups of the *N*-acetyl-*d*-glucosamine residues are removed. Chitosan-based materials show various interesting properties, which make them pertinent in many fields, including agriculture, where they are used as plant biostimulants. Chitosan induces several defensive genes in plants, such aspathogenesis-related genes, like glucanase and chitinase. Likewise, it induces many enzymes in the reactiveoxygen species scavenging system, such as superoxide dismutase, catalase and peroxidase. The signal transduction pathway from chitosan that elicits its responses includes hydrogen peroxide and nitricoxide signals, and it may also directly control gene expression by interacting with chromatin. Chitosan has been applied both as a plant biostimulant to improve plant growth, and abiotic stress and pathogen resistance. However, these responses are complex and they depend on different chitosan-based products and concentrations as well as the plant species and growth stage [59].

A-6) Phytohormones

Organic compounds of phytohormones, except for nutrients, are called biostimulants or bioinhibitors. They regulate plant physiological processes and stimulate or inhibit specific enzymes inside the cells and modify plant metabolism in low concentrations [60]. Since 1937, auxin, gibberellin (GA), ethylene, cytokinin, and abscisic acid (ABA) have been called phytohormones, or “classical five” (Figure 1). This group is expected to grow as the hormonal functions of other compounds are recognized and as new hormones are discovered. The natural auxin in plants is indole-3-acetic acid (IAA) [61].

B) Synthetic bio-stimulants are composed, among others, of plant growth regulators (synthetic hormones), phenolic compounds, inorganic salts, essential elements, and different substances that have stimulating properties for plants.

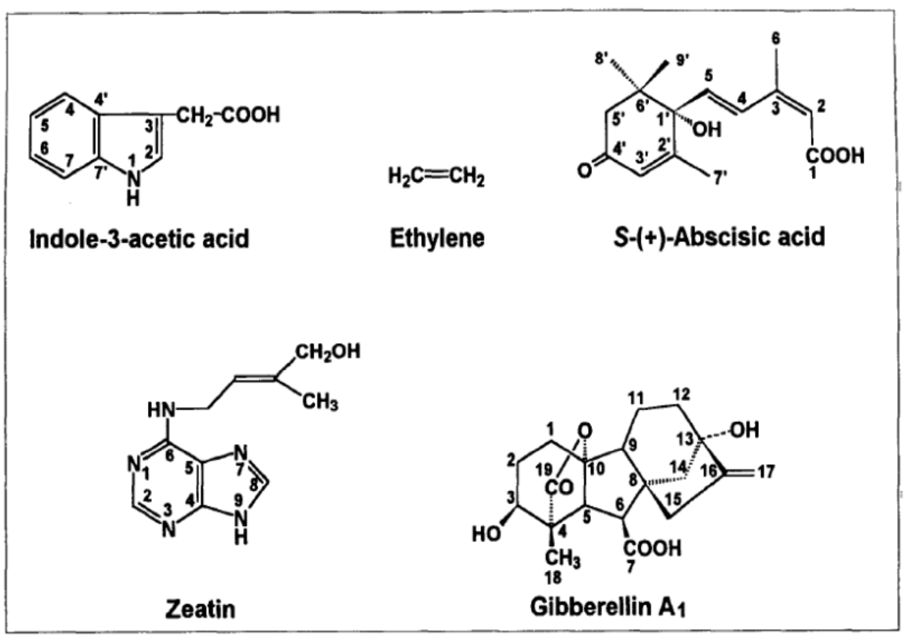

Figure 1- Structure of 5 classical hormones

B-1) Phosphite

Over the past three decades, phosphite (Phi; H_2PO_3) or its conjugate phosphorous acid (H_3PO_3), a reduced form of Pi and inorganic salt, has significantly been used as a pesticide, supplemental fertilizer, and plant biostimulant. As a plant biostimulant, Phi has been proved to improve nutrient uptake and assimilation, abiotic stress tolerance and product quality. Moreover, Phi improves root growth, yield and nutritional valueof horticultural crops. Furthermore, Phi is largely used for controlling pathogens and in many countries it is registered as a fungicide and bactericide. Though this Pi analogue is used as an alternative fertilizer, its contribution to P nutrition is limited and it has been the subject of discussion. The extensive application of Phi and its related products in agriculture has brought considerable debate in the technical and scientific world [62, 63].

B-2) Selenium (Se)

In spite of no need to Se for higher plants, there are reports about beneficial effects of Se at low concentrations on plant growth especially in hyper accumulators, which can reach two fold higher biomass in the presence of Se [64]. Selenium as almost essential micronutrient improves different physiological and biochemical traits. It exists in all environments as a natural metalloid element and is considered as a limited and non-renewable resource on the Earth. It can be found at very high levels in alkaline soils where Cretaceous shale or other seleniferous rocks are the soil parent material [65, 66].

Some of elements such as Al, Co, Na, Se, and Si are called beneficial elements as they improve growth and may be essential to particular taxa but are not required by all plants. These elements enhance growth for various taxa under certain environmental conditions, however, the effect and concentration is different for each element and plant species [67].

B-3) Plant growth Promoting Products (Synthetic hormones)

A wide category of plant growth promoting products are being applied for positive effects on crop growth and yield. The ingredients in these products are such as: Adenine, AMP and cyclic AMP, Indole butyric acid, Polyethylene glycol, Dinoseb, Carboxylic and phenolic acids [60].

Plant biostimulants applicationin medicinal plants

There are some reports on positive effects of biostimulants application on medicinal plants.In growing the medicinal plants, it is vital to associate the biomass production to quality of the raw material. The application of biostimulants in the commercial production of medicinal plants is a viable management practice for the production of these species, increasing biomass production and enhancing secondary metabolites synthesis. Studies about the effect of plant biostimulants on the accumulation of secondary metabolites in medicinal plants have been conducted in order to increase the medicinal and trade values of these species [68]. The development of biostimulants may follow a classical ‘pharmacological’ approach, where candidate active substances or microorganisms are screened in controlled conditions and a stepwise procedure is followed for selecting promising candidates, moving from the laboratory to more realistic conditions.

In *Anethum graveolens* L. plants, morphological traits of umbel number per plant, dry weight of plant, seed weight per plant were improved by seed inoculation and foliar application of vermicompost and biostimulant mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum* [69] (Table 7). Some other studies have reported the positive effect of these biostimulants on other medicinal plants such as coriander [70], celery [71], fennel [33], turmeric [72] and hyssop [73, 74] (Table 7). El-Sharabasy et al. (2012) reported about amino acids significant influence on the synthesis of secondary metabolites. They expressed that the steroid biosynthesis have a positive correlation with increasing the glutamine concentration, so that the highest steroid content was recorded in the application of glutamine at 500 $mg.l^{-1}$ [75] (Table 7). Findings of Ardabili et al. (2012) research demonstrated that foliar application of Aminolforte increased content of antioxidant enzymes, peroxidase, poly phenol oxidase, phenylalanine ammonia lyase, phenol contents and other phytochemical traits in *Aloe vera* plants [76] (Table 7). Foliar application of various plant biostimulants such as spermidine and stigmastrol, vermicompost, ornithine, proline and phenylalanine and Amino total had positive effect on morphological and phytochemical traits of *Matricaria chamomile* L. from Asteraceae family [77-80] (Table 7), while foliar application and seed treatments of humic acid, Trans-cinamic acid and plantbiostimulants formulations of Aminolforte, Kadostim, Fosnutren and Humiforte demonstrated the significant effect on *Ocimum basilicum* L. plants from Lamiaceae family [81-84]. Furthermore, the others recorded the cytokinin (90 $mg.l^{-1}$), auxin (50 $mg.l^{-1}$) and gibberellic acid and also four commercial formulations Aminolforte, Kadostim, Fosnutren and Hmiforte as the best plant biostimulant for improvement of morphological and phytochemical traits of *Calendula officinalis* L. from Asteraceae family [85-105] (Table 6-8).

Mechanism of action and types of effects of plant biostimulant

It is impossible to suggest one common mode of action for all biostimulants, but researchers suggest that the effect of biostimulants on plants is a consequence of their influence on plant metabolism in a wide sense of the word. They stimulate the synthesis of natural hormones, sometimes increasing their activity, facilitate the uptake of nutrients from the substrate, stimulate root growth, and cause higher yield, often improving its quality at the same time. Moreover, they increase the resistance of plants to unfavorable conditions such as drought, frost, pollution of the environment with heavy metals, etc. This may be related to changes in enzymatic activity and the synthesis of antioxidants. They may enhance water-holding capacity, increase antioxidants, and induce metabolism. According to a Dutch producer (personal contacts), plant biostimulants are agents which at very low concentrations enhance the basic biochemical processes in plants and soil, and thereby improve the growth and development of plants, and increase their resistance to stress. Plant biostimulants are not a substitute for fertilizers, manure or other sources of mineral nutrients [106, 9].

A) Nitrogen assimilation

Recent studies suggest that active molecules contained in plant biostimulants can advance nitrogen assimilation through stimulation of the activity and transcription of N assimilation and Krebs' cycle enzymes [107-111]. Bacteria can have positive effects

Table 7- The effect of different formulations and treatments of biostimulants on the different traits of medicinal plants)

Best treatments of Biostimulants	Place	Species	English name	Application	Result - Improve and enhance (up to)	Reference
•Cytokinin (90 $mg.L^{-1}$), auxin (50 $mg.L^{-1}$) and gibberellic acid (50 $mg.L^{-1}$)	Field	*Calendula officinalis* L.	Marigold	Foliar application	•Morphological traits: height (12cm), number of leaves (7num.), shoot dry matter (3.0 g), root dry matter (0.6 g), total dry matter (4.0 g), number of flower heads (1.0 num.), Phytochemical traits: Flavonoid content (1.42 $g.100g^{-1}$)	Vivan et al.,2014 [68]
•Vermicompost (8 and 12 $ton.ha^{-1}$) and mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum*	Field	*Anethum graveolens* L.	Dill	Inoculated seeds and inoculated seeds + spray on the plant base at stem elongation stage	•Morphological traits: Umbel number per plant (14), dry weight of plant (21.08 g), seed weight per plant (8.4 g)	Darzi et al., 2012 [69]
•Vermicompost (in level of 6 $ton.ha^{-1}$)and mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum*	Field	*Coriandrum sativum* L.	Coriander	Seed inoculation	•Essential oil and its components: essential oil content (0.48%), essential oil yield (13.84$kg.ha^{-1}$) and alpha pinene percent in essential (4.76%)	Shirkhodai et al., 2014 [74]
•Aminolforte (0.1 and 0.15% v/v)	Greenhouse	*Aloe vera*	*Aloe vera*	Foliar application	•Phytochemical traits: antioxidant enzymes (22%), peroxidase (0.035 $\Delta Amin^{-1}\mu g^{-1}pr$), poly phenol oxidase (0.1 $UnitE.\mu g^{-1}pr$), phenylalanine ammonia lyase (0.12$\mu gCin.\ \mu g^{-1}pr$) , phenol contents (18 $\mu g.g^{-1}fw$), alkaloid contents (2 $oDg^{-1}dw$), total soluble carbohydrates (1.7 $mg.g^{-1}fw$), ascorbate (35 $\mu Molg^{-1}fw$) , reduced glutathione (42 $\mu Molg^{-1}fw$)	Ardabiliet al., 2012 [76]
•Vermicompost(20 $ton.ha^{-1}$) Fosnutren (at budding + flowering stage)	Field	*Matricaria chamomilla* L.	Chamomile	Foliar application	•Plant height (38.7 cm), flower head diameter (20.8 mm), fresh flower yield (2868.09 $kg.ha^{-1}$), dry flower yield (572.15 $kg.ha^{-1}$) Esssential oil percent (0.39%)	Hajseyed hadiet al., 2011 [78]
•Humic acid with and without PGPR	Field	*Ocimum basilicum* L.	Basil	Seed treatments and foliar application	•Yield of oil	Behrozfar et al., 2013[81]
•Chitosan (3 $cm.L^{-1}$), amino acid (3 $cm.L^{-1}$), seaweed (3 $g.L^{-1}$)	Field	*Allium sativum* L.	Garlic plants	Foliar application	•Morphological traits: plant height (68.5 cm), leaves number (8.75 no.), fresh weight of leaves (90.34 $g.plant^{-1}$), neck (18.54 $g.plant^{-1}$) and bulb (54.37 $g.plant^{-1}$), dry weight of leaves (18.26 $g.plant^{-1}$), neck (6.43$g.plant^{-1}$) and bulb (16.86 $g.plant^{-1}$), bulb diameter (3.88 cm), total yield (5.51 $ton.fed^{-1}$), Biochemical traits: Fe (0.86 ppm), Zn (14.65 ppm), Cu (14.56 ppm), Mn (17.43 ppm)	fawzy et al, 2012 [88]
•Mycorrhizal fungi *Glomus etunicatum* and *Glomus mosseae*	Greenhouse	*Mentha spicata*	Mint	Fungal inoculation	•Biomass amount (dry weight), essential oil concentration, total shoot and root, phenolic compounds, shoot anthocyanin, cinnamic acid content	Bagheri et al., 2014 [89]

Table 7- Continued

Biostimulants	Place	Species	English name	Application	Result - Improve and enhance (up to)	Reference
•Ornithine (50 $mg.L^{-1}$), proline (100 $mg.L^{-1}$)and phenylalanine (100 $mg.L^{-1}$)	Pot	*Matricaria chamomila* L.	Chamomile	Foliar application	•Morphological traits:plant height (31.3 cm), number of branches (19 no.), number of flowers per plant (53.3 no.), herb fresh weight (38.94 $g.plant^{-1}$) and herb dry weight (4.59 $g.plant^{-1}$) and flowers fresh weight (4.94 $g.plant^{-1}$), flowers dry weight (0.94 $g.plant^{-1}$) Essentiaol oil percent (0.79%) and yield (388.9 ml/flower heads/plant), Farnesene (9.48%), α-BisobololB (7.43%), oxide α-Bisobolol (14.84%) Phytochemical traits: Total phenol (25.8 $mg.g^{-1}$) and total indole (11.0 $mg.g^{-1}$)	Karima and Abd-El-Vahed 2005[79]
•Biostimulators of Kadostim, Humiforte (each of them 1.5 $L.ha^{-1}$)	Field	*Calendula officinalis* L.	Pot marigold	Foliar application	•Morphological traits: Capitula dry weight (46 $g.m^{-2}$), leaves dry weight, total dry weight (161.2 $g.m^{-2}$), Phytochemical traits: total carbohydrates of capitula (0.28 $mg.g^{-1}DW$), total carbohydrates in leaves (0.24 $mg.g^{-1}DW$), N (2.07%), P (1.11 $mg.g^{-1}DW$), K (2.13 $mg.g^{-1}DW$), Fe, Zn, Cu, Ca	Rafiee et al., 2013 [86]
•Amino total (1.2 $ml.l^{-1}$), yeast (2 $g.l^{-1}$)	Field	*Allium sativum* L.	Garlic	Foliar application	•Morphological traits: plant height (90 cm), leaf number (11no.), total yield (9.8 $ton.fedd^{-1}$), bulb weight (72 g), bulb diameter (4.5 cm), bulbing ratio, outer cloves (9.6 no.), total cloves (18.7 no.)	Salary and El-Ramady, 2014[90]
•Aminolforte,Kadostim, Fosnutren, and Humiforte (each of them 2 $L.ha^{-1}$)	Field	*Melissa officinalis* L.	Lemon Balm	Foliar application	•Morphological traits: plant height (64.5 cm), number of flowers per stem (55.3 no.), SPAD value (32.83 SPAD), leaf length (52.6 mm), leaf area (60.2 cm^2), leaf number (74), leaf dry weight (1.93 $t.ha^{-1}$), shoot dry weight (2.89 $t.ha^{-1}$), number of branches per plant (8 no.) Essential oil and its components: Citranellal (4.23%), neral (33.12%), deltacadinene (3.76%), germacrene (2.89%), geranial (26.86%).	Mehrafarin et al., 2015 [91]
•Mycorrihza fungi inoculation under salt stress	Field	*Rosemarinus officinalis* L.	Rosemary	Inoculation	•Morphological traits: number of shoots per plant, number of leaves per plant, stem diameter, leaf width, stems fresh weight, roots fresh and dry weight, Essential oil and its components	Bahonar et al. 2015 [92]
•Panchakavya (2% and 4%), moringa leaf extract (2 and 4%) and humic acid (0.2 and 0.4%)	Field	*Cassia angustifolia* var. KKM.1)	Senna	Foliar application	•Morphological traits:plant height (35.9 cm), number of branches, number of pods, dry leaf yield, dry pod yield	Davary panah and Farah vash, 2014[93]
•Methanol (15 and 30 %v/v) and Aminolforte, Kadostim (at 1.5 liters per hectare)	Greenhouse	*Satureja hortensis* L.	Savory	Foliar application	•Morphological traits: Leaf fresh (0.66 $g.plant^{-1}$) and dry (0.29 $g.plant^{-1}$) weight, shoot fresh (1.63 $g.plant^{-1}$) and dry (0.59 $g.plant^{-1}$) weight, number of leaves per plant (72), number of flowers per plant (6 no.) , chlorophyll content (31.16 SPAD)	Mehrabi et al.,2013 [94]
•Tyrosine and zinc(100 ppm)	Greenhouse	*Salvia farinaceae*	Blue sage	Foliar application	•Morphological traits:Plant height, number of leaves and branches, fresh and dry weight of (leaves, branches, shoots)and stem diameter, length of peduncle, length of main inflorescence, number of inflorescence and florets, andfresh and dry weight of inflorescences/ plant Phytochemical traits: Content of chlorophyll a, total chl. and total caroteniodes in leaves, soluble sugars in leaves	Abd el Aziz and Balba, 2007 [95]

Table 7- Continued

Biostimulants	Place	Species	English name	Application	Result - Improve and enhance (up to)	Reference
•Amino total (125 - 375 ppm) in salinity conditions	Field	*Matricaria recutita* L.	Chamonile	Foliar application	•Plant height (56 cm), number of branches (50 no.), fresh and dry flower yield,chlorophyll a (8.67 $mg.g^{-1}$), chlorophyll b (1.27 $mg.g^{-1}$), and carotene content (2.9 $mg.g^{-1}$), proline (1.50 $mg.g^{-1}$) and polyphenol (537.89 $mg.g^{-1}$) content, content of total flavonoids (370.2 $mg.g^{-1}$) Essential oil % (0.54%) and yield (2.66 $L.fed^{-1}$), α-bisabolol oxide A, α-bisabolol oxide B, cis-β-farnesene, and bisabolone oxide	Omer et al., 2013 [80]
•Kadostim (1.5 $L.ha^{-1}$)× Nitroxin(0.5 $L.ha^{-1}$) and Fosnutren (1.5 $L.ha^{-1}$) × Nitroxin (0.5 $L.ha^{-1}$)	Greenhouse	*Ocimum basilicum* L.	Basil	Foliar application and seed inoculation	•Plant height (80.15 cm), secondary stem number (16.08 no.), leaves number (128.17 no.), total dry weight (16.97 $g.plant^{-1}$), stem diameter (6.33mm), flower dry weight (11.73 $g.plant^{-1}$)	Rahimi et al., 2013 [82]
•Kadostim (1.5 $L.ha^{-1}$)× Nitroxin (0.5 $L.ha^{-1}$) and Fosnutren (1.5 $L.ha^{-1}$) × Nitroxin (0.5 $L.ha^{-1}$)	Greenhouse	*Ocimum basilicum* L.	Basil	Foliar application and seed inoculation	•Leaf fresh (25.47$g.plant^{-1}$) and dry (6.48 $g.plant^{-1}$) weight, leaf number (99 no.), leaf area (902.3 $mm^2.plant^{-1}$) Essential oil content (0.43%), methyl chavicol (37.13%), geranial (29.05%), caryophelene (6.66%), carvacrol (31.22%)	Rahimi Shokouh et al., 2013 [83]
•Kadostim, Humiforte (each of them 1.5 $L.ha^{-1}$)	Field	*Calendula officinalis* L.	Marigold	Foliar application	•Plant height (13.41 cm), leaves number (21no.), plant dry weight (161.2 $g.m^{-2}$), capitula dry weight (46 $g.m^{-2}$), leaf area (492.3 mm^2), relative water content (88.58 RWC), harvest index (9.8%), capitula number per plant (380 no.), Phytochemical traits: total carbohydrates of capitula (0.28 $mg.g^{-1}$DW) and leaves (0.24 $mg.g^{-1}$DW) , total flavonoids of leaves (0.1%) and capitula (0.24%), yield of flavonoids in capitula (0.80 $g.m^{-2}$) and leaves (0.021 $g.m^{-2}$), N (2.07%), P (1.11 $mg.g^{-1}$DW), K (2.13 $mg.g^{-1}$DW).	Rafiee et al., 2015 [87]
•Amino acids (tyrosine and phenylalanine) and phenolic acids (trans-cinnamic acid, benzoic acid and salicylic acid)	Pot	*Ammi visnaga*	Khella	Foliar application	•Shoot height (120 $cm.plant^{-1}$), fresh (75 $g.plant^{-1}$) and dry (51 $g.plant^{-1}$)biomass, number of branches and number of umbels per plant (40 no.), seed yield Essential oil content and components: 2,2-dimethyl butanoic acid, isobutylisobutyrate,α-isophorone, thymol, fenchyl acetate and linalool	Iman et al., 2013 [96]
•Aminolforte 20	Field	*Camellia sp.*	Tea	Foliar application	•SPAD values (3.75 $mg.g^{-1}$)(fr.wt.), total polyphenols (24.38%) and amino aicds (4.43%), catechins (17.24%), theaflavins, thearubigins, total liquor color, colour index, caffeine,	Thomas et al., 2009 [97]
•Pepton (at 0.5%) in combination with mixture of some chelated micro elements (at 0.25% Zn + Mn + Fe)	Field	*Olea europaea* L.	Olive tree	Foliar application	•Morphological traits: height (96.33 cm) of the plant, leaves number (124 no.) and leaves area (949 cm^2) Phytochemical traits: macro elements (1.4 ppm N;0.8 ppm K), trace elements (800ppm Fe; 200 ppm Zn)	Yousef et al., 2011 [98]

Table 7- Continued

Biostimulants	Place	Species	English name	Application	Result - Improve and enhance (up to)	Reference
•Aminolforte and Fosnutren (1.5 $L.ha^{-1}$)	Field	*Rosemarinus officinalis* L.	Rosemary	Foliar application	•Plant height, collar diameter of stem, num. of leaves per plant, leaf length, num. of branches per plant, Stem dry weight, stem fresh weight, leaf fresh weight, total fresh weight, leaf dry weight, total dry weight, SPAD value, Essential oil,α-pinene, canphore	Foroutan nia et al., 2014 [99]
•Kadostim, and Humiforte (each of them 1.5 $L.ha^{-1}$) Filed	Greenhouse	*Calendula officinalis* L.	Pot marigold	Foliar application	•Capitula (44.0 $g.m^{-2}$), leaves (25.24 $g.m^{-2}$) and total (165.81$g.m^{-2}$) dry weight of the plant, total carbohydrates of capitula (0.28$mg.g^{-1}$DW) and leaves (0.21 $mg.g^{-1}$DW) , total flavonoids of capitula (0.25 $mg.g^{-1}$DW)and leaves (0.1 $mg.g^{-1}$DW), N (1.98%), P (1.04 $mg.g^{-1}$DW), K (2.13 $mg.g^{-1}$DW), Fe (2.80 $mg.g^{-1}$DW), Zn (0.58 $mg.g^{-1}$DW), Cu (0.31 $mg.g^{-1}$DW), Mn (2.20 $mg.g^{-1}$DW) , Ca (24.50 $mg.g^{-1}$DW)	Rafiee et al., 2015 [100]
•Trans-cinamic acid (250 $mg.l^{-1}$) and putresine (150 $mg.l^{-1}$)	Greenhouse	*Ocimum basilicum* L.	Basil	Foliar spray	•Plant height, num. of branches per plant, fresh weight of herb, dry weight of herb, Essential oil content and its components, oil yield, total carbohydrates, total soluble sugars, total nitrogen, P, K, Fe, Zn	Talaat and Balba, 2010 [101]

Table 8- Reports of defense elicitation in medicinal plants using polysaccharides from macroalgae

Elicitor	Plant	Results	Referemce
Alginate	Tobacco	PAL activity and resistance to TMV	Laporte et al., 2007 [102]
Carrageenan	Chickpa	Secondary metabolites	Bi et al., 2011 [103]
Irradiated carrageenan	Fennel	Essential oils fenchone and anethole	Hashmi et al., 2012 [104]
Irradiated carrageenan	Field mint	Essential oils and menthol	Naeem et al., 2012 [105]

on plants through several mechanisms; maintaining soil fertility, nitrogen fixation, improving root elongation, solubilizing insoluble minerals through the production of acids, increasing the absorption of phosphorus and other elements in soil [112]. Free-living nitrogen fixing bacteria such as; *Azotobacter chroococcum* and *Azospirillum lipoferum*, were found to have the ability to fix nitrogen as well as the ability to release phytohormones like gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis [32, 33]. Therefore, N_2-fixing and phosphate solubilizing bacteria, including *Bacillus* sp. are extensively applied in organic plant growing [113].

B) Modification and mobility of PGRs

Biostimulants effect may be attributed to the mobility of PGRs within the plant, the potential for signal enhancement and the ability to promote complex regulatory actions through interactions between different biochemical and physiological reactions [114]. However, thereis a high variability in the effects of PGRs or biostimulants on plants, depending on the crop,environment and agricultural practices. The regulatory effect of amino acids on growth could be clarified by this theory that some amino acids can affect through their influence on gibberellins biosynthesis [115]. Plant biostimulants can modify the hormonal status of a plant and exert influence over itsgrowth. The obtained results were because of the biostimulant potential effects as hormonal nature by providing plant hormones, enhancing hormonal activity direct supply of plant hormones [87]. Active dry yeast is a natural safety biofertilizer. It is considered as a natural source of cytokinins that increases cell division and enlargement as well as the synthesis of protein, and nucleic acid [112].

C) Induction of the metabolic pathway

The induction of the metabolic pathway associated with the synthesis of phenylpropanoids in plants treated by biostimulants may clarify why these products can help plants to overcome stress conditions [116,117]. Specifically, a protein hydrolyzate based fertilizer obtained from alfalfa hydrolyzate plants (AH) was demonstrated to help maize plants to overcome salinity stress through stimulation of enzymes functioning in nitrogen metabolism, increase of phenylalanine ammonia-lyase (PAL) activity and transcription, anincrease of flavonoid synthesis [118,112,119]. PAL is a vital enzyme that catalyzes the first committed step inthe biosynthesis of phenolics by changing phenylalanine to trans-cinnamic acid and tyrosine to p-coumaric acid, opening the way to the secondary metabolism. The biostimulant activity of AH was identified with thepresence of triacontanol (TRIA) and indole-3-acetic acid (IAA), two important regulators of plant metabolism [18]. Indeed, biostimulants can induce the activity and gene expression of several enzymes involved in the tricarboxylic acid cycle (TCA), as previously observed in maize plants treated with an alfalfa based-hydrolizate [119]. Furthermore, amino total as a source of amino acids may play an important role in plant metabolism and protein assimilation which are fundamental for cell formation and consequently increase fresh and dry biomass. This regulatory effect was observed on strawberry [120], and celery [121].An oxidative burst response, with hydrogen peroxide (H_2O_2)synthesis, has been found in numerous plants treated with chitosan [122, 123]. This led to the induction ofplant defense enzymes, including phenylalanine ammonia-lyase(PAL), which is a key enzyme in phenolic compound biosynthesis [124]. The induction of PAL by chitosan correlated well with the accumulation of phenolic compounds afterchitosan treatment in many plant species, such as papaya [125, 126] and sweet basil (*Ocimum basilicum* L.) [127]. Humic acids have been appeared to stimulate plant growth and yield by inducing the mechanisms involved in: cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities, improvement of soil structure and increase of microbialpopulations[119]. Algae extract as a new biofertilizer containing N, P, K, Ca, Mg, and S and in addition, Zn, Fe, Mn, Cu, Mo, and Co, some growth regulators, polyamines, natural enzymes carbohydrates, proteins and vitamins was applied to enhance vegetative growth and yield [66]. The effect of active dry yeast on plant growth may be attributed to its content of different nutrients, higher amount of proteins and vitamins, especially vitamin B which may play an important role improving growth and controlling the incidence of fungal diseases, as mentioned by Bevilacqua et al., 2008 [128].

The mechanisms behind the physiological and biochemical effects of biostimulants on plants are frequently obscure, because of the heterogeneous nature of the raw materials used for their production. Moreover, these effects are often the result of many components that may work synergistically in various ways.

D) Signal transduction on metabolic pathways

Plant biostimulants may influence plant secondary metabolism by modulating the rates of biosynthesis, accumulation and/or vacuolar transport, turnover and degradation [129]. It is feasible that elicitation modulates the expression of molecules of primary metabolism and subsequently regulates the content of secondary metabolites. One of the best studied responses to stress in plants is the biosynthesis of phytoalexins, secondary metabolites of low molecular weight with antimicrobial effects. The application of different abiotic or biotic biostimulants can trigger the synthesis and accumulation of these compounds [130]. Plants have the ability to recognize a number of structurally distinct molecules as signals and could be clarified by the presence of specific receptors for each elicitor class. Signal perception is the initial step of the elicitor signal transduction cascade and, for instance, recognition of different stimuli is central to the ability of plants to respond through activation of kinases, synthesis of reactive oxygen species, ion fluxes and cytoplasm acidification [131, 132]. Plant G-proteins have been involved in different cellular processes linked to growth, hormone signaling, growth and defense responses [133, 134], and an enhancing body of knowledge has involved G-proteins in the response to biostmulants. There are observations confirming that the PLC/IP3-DAG/ PKC pathway occurs in plants [135-140]. Several of these studies showed that this cascade plays a role in the responses of plants to elicitors [141]. CAMP levels have been appeared to increase in response to elicitor treatment and mediate the stimulation of phytoalexin biosynthesis in some species, such as carrot [142], lucerne [143], French bean [144] and Mexican cypress cell cultures [145].Calcium is a ubiquitous signal in plants which mediates the regulation of many cellular processes by different stimuli, among them, elicitation. There is evidence that the action of many biostimulants includes changes in the intracellular calcium status. Recently, it has been demonstrated that phosphoinositide 3-kinase likewise plays a role in elicitation-mediated secondary metabolite synthesis. Thus, the increase in the synthesis of anthraquinone in *R.tinctorum* L. induced by chitosan is intervened by PI_3K activity [146], which in turn stimulates MAPK. MAPK cascades are major components downstream of receptors/sensors that transduce external signals into intracellular reactions in all eukaryotes. Plant MAPKs are activated by an assortment of biotic and abiotic stimuli, including pathogen attack, wounding, temperature, drought, salinity, osmosis pressure, UV irradiation, ozone and reactive oxygen species [150]. MAPK activation generally induces specific gene expression. Laboratory researches have shown that the final steps in chitosan signaling are mediated by translocation of MAPK to the nucleus [147]. In turn, MAPK phosphorylates transcription factors, which results in improved expression of genes coding for enzymes, will play a basic role in the biosynthetic pathway of secondary metabolites such as phytoalexins [148, 149] (Fig. 2, 3).

Plant biostimulants application in tissue and cell cultures

Tissue cultures of *Alternanthera brasiliana* (L.) Kuntze were treated with various growth regulators (Kinetin and 2,4-D), tyrosine and ultraviolet A radiation (UV-A; 320 - 400 nm) to study their effects on growth and pigment synthesis. Nodal sections of plantlets grown from seeds were inoculated in all tested media and different light conditions. After eight weeks, this material was used to evaluate biomass, chlorophyll and betacyanin production. The Murashige and Skoog (MS) + kinetin medium caused growth of approximately four shoots/explant. This medium in addition to white light was the best combination for micropropagation with the highest root growth and betacyanin synthesis. Plantlets grown under UV-A illumination decreased biomass weight and worse Chlorophyll *a* / Chlorophyll *b* ratio. Addition of 2,4-D brought about inhibition of pigment synthesis and growth of plantlets [150].

Studies were conducted for the effect of amino acids and growth regulators on the production of artemisin, an antimalarial compound, in the callus of *Artemisia absinthium*. Callus was initiated on solid MS medium supplemented with BAP and NAA from leaf explant. For the production of artemisinin, the callus was proliferated on sterile filter paper bridge in MS medium supplemented with different concentrations of plant growth regulators and amino acids. Estimation of artemisinin content showed that leaves contained 223 $\mu g.g^{-1}$ artemisinin while no artemisinin was observed in the stem extract. Callus culture initiated from leaf explant on MS medium with no growth regulator, did not show the presence of artemisinin. The amount of artemisinin in the callus culture was affected by the addition of various growth regulators and amino acids to the medium; 3.1 $\mu g.g^{-1}$ artemisinin was present in the callus cultured on MS medium to which

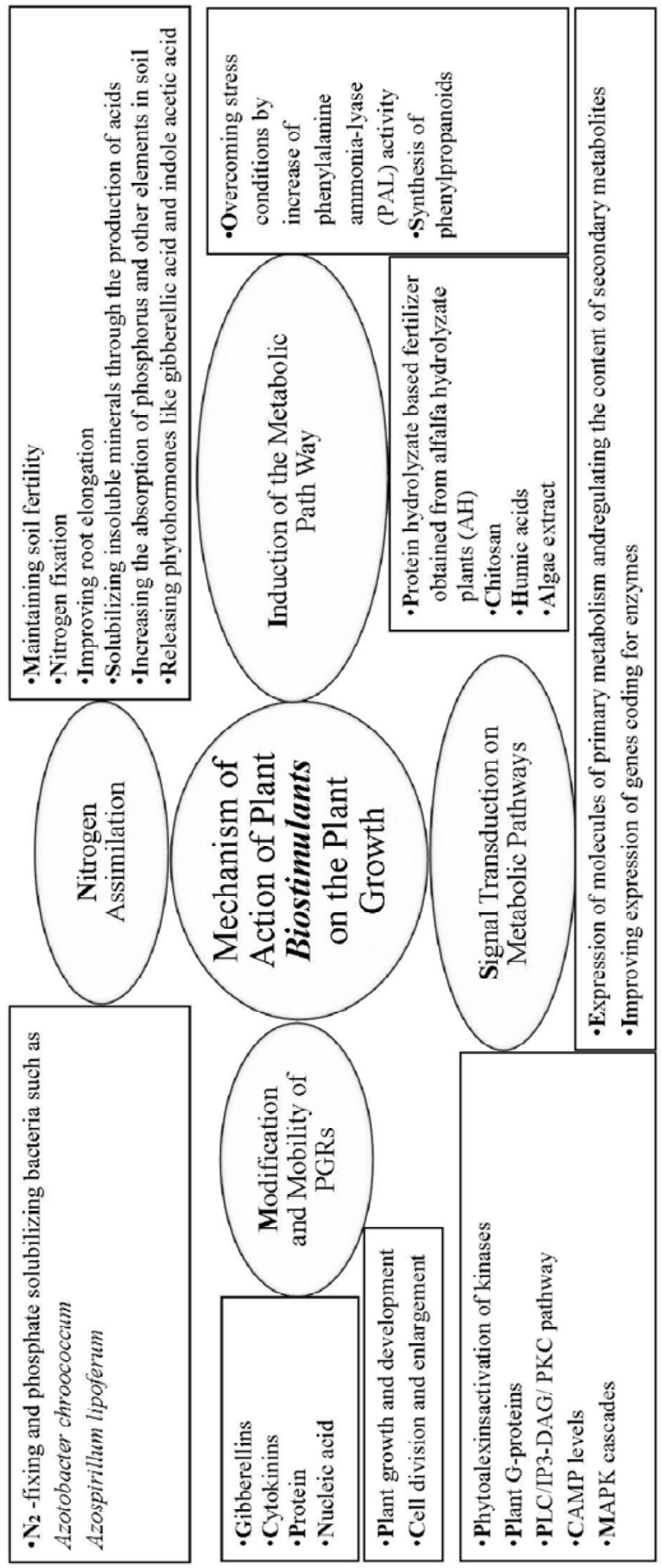

Figure 2- Schematic representation of the main effects and physiological actions elicited by plant biostimulants (PBS) and possible mechanism(s) of action

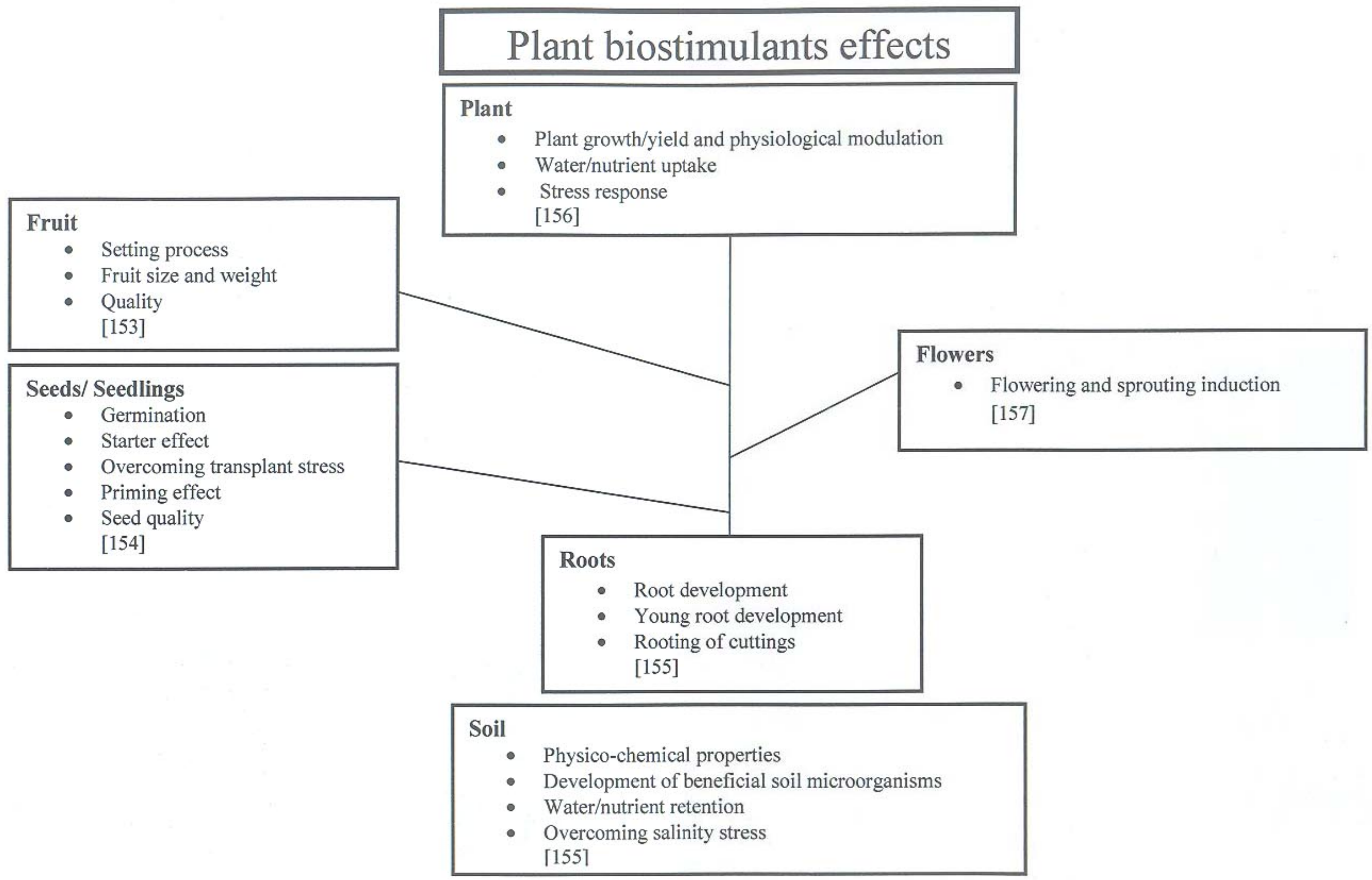

Figure 3- Reported examples of the main effects and physiological actions played by plant biostimulants (PBS) [158]

valine (12.5 mg.l^{-1}) was added. Addition of cystine (12.5 mg.l^{-1}) to the medium resulted in 2.8 µg.g^{-1} artemisinin production. The amount of artemisinin in the callus cultures was 3.05µg.g^{-1} and 1.95 µg.g^{-1} when BAP (2 mg.l^{-1}) and NAA (2 mg.l^{-1}), respectively were available in the medium. Addition of other growth regulators and amino acids resulted in no artemisinin synthesis. Present study suggests that artimisinin production can be improved by the control of medium by different hormones and amino acids in the callus cultured on sterile filter paper bridge [151] (Table 9).

Propagation of high-quality seedlings by tissue culture with application of seaweed extract as biostimulants was conducted as a research instead of synthetic chemicals. The nodal explant of field grown *W. somnifera* estabilised on Murashige and Skoogs medium (MS) and Gamborg B_5 medium supplemented with six concentration of 2,4-D. The percentage of culture response from the nodal explant ranged from 44 to 80 and 3.0 mg l-1 2,4-D found to be best for callus induction. MS media containing different concentration of seaweed extract (10, 20, 40, 60, 80, and 100 %) were tested individually for shoot induction. The medium supplemented with 40 % seaweed extract exhibited maximum number of shoots with about 8.6 shoots/ callus and 80 % seaweed extract exhibited 4.3 shoots/ callus. It is evident from this study that seaweed extracts can be used as substitute for synthetic growth hormones for micropropagation of medicinally important plant *W. somnifera* for clonal propagation and conservation [47].

Table 9- Secondary metabolite production by cell cultures of medicinal plants [160]

SI. No.	Secondary metabolite	Plant	Biostimulants/Elicitor
1	5'-phosphodiesterase (PDase)	*Catharanthus roseus*	*Alteromonas macleodii,* Alginate oligomers
2	Acridone expoxide	*Ruta gravelones*	Fungal poly saccharide
3	Ajmalicine	*Catharanthus roseus*	a) *Pythium* sp. b)Yeast elicitor, MeJA c) *Trichoderma viride* *d)Pythium aphanidermatum* e) Jasmonic acid
4	Alkaloids (tropane)	*Datura stramonium*	*Phytopthora megasperma*
5	Anthraquinones	*Morinda citrifolia*	Chitin 50
6	azadirachtin	*Azadirachta indica*	Jasmonic acid, salicylic acid
7	Berberine	*Thalictrum rugosum*	*Sacharomyces cerevisiae*
8	Capsidiol	*Capsicum annum*	*Trichoderma viride*
9	Capsidiol, debneyol, scopoletin, nicotine	*Nicotiana tabacum*	*Phytopthora cryptogea* Yeast extract Cryptogein Cellulase, MeJA
10	Codeine, morphine	*Papaver somniferum*	Fungal spores
11	Diosgenin	*Dioscorea deltoida*	*Rhizopus arrhizus*
12	Diterpenoid tanshinones	*Salvia miltiorrhiza*	Yeast elicitor
13	Glyceollins, apigenin, genistein, luteolin	*Glycine max*	β-glucan, MeJA
14	Hyoscyamine, scopolamine	*Hyoscyamus niger, H. muticus*	Fungal elicitor, MeJA
15	Indole glucosinolates, Camalexin	*Arabidopsis thaliana*	Fungal, MeJA
16	Isoflavonoids	*Lotus corniculatus*	Glutathione
17	Kinobeon A	*Carthamus tinctorius*	Blue green algae
18	Methoxymellein, 4-hydroxybenzoic acid	*Dacus carota*	Fungal elicitor
19	Raucaffrincine	*Rauwolfia canescens*	Yeast elicitor, MeJA

Effect of foliar application of biostimulants on developmental biology of the potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) was compared with untreated plants. Neonate larvae mortality on biostimulant treated foliage was greater than that of larvae confined to untreated foliage. No significant differences in feeding parameters were observed between larvae fed on treated potato foliage and untreated plants. However, according to the host suitability index (HSI), biostimulant-treated plants became less suitable for PTM development. Biostimulant applications significantly increased the number of hairs and trichomes per unit potato leaflet surface suggesting that neonate's mortality, or larval failure of initial establishment, may have been an outcome of a higher number of hairs and trichomes associated with biostimulant-treated plants. From a horticultural perspective, the organic biostimulant significantly increased tuber yield per plant and induced early maturity in potato plants by 20.9 and 19.6% above the control, respectively [152].

Some of plant biostimulants in Iran

The past years have witnessed increasing growth in the use of biostimulants in Iran agriculture. Such Products, except for fertilizers, crop protections and crop nutritions, have attracted the attentions in the Research centers, manufacturers and companies in Iran. Some products of companies active in formulation of biostimulants in Iran are mentioned in Table 10.

Conclusion

Biostimulants as effective substances, environmentally friendly products and safe for human beings are being extensively applied in agriculture and horticulture. Yield response in medicinal plants to biostimulants application showed that each medicinal plant responds in a different way based on the chemical composition, components of biostimulants, and the timing and rate of application. However, more information about these procedures are needed and this topic needs more study and investigation. On the basis of the information described in the present review, some points are represented as conclusion.

1- Introduction of new formulations of biostimulants in production of medicinal plants by aim of increasing secondary metabolites quality and quantity.

2- In this review a category of the biostimulants is represented based on natural and synthetic components. As a new approach it is recommended to categorize these formulations based on their most significant effect on medicinal or the other plants.

3- About biostimulants mode of action three categories of nitrogen fixing bacteria, biostimulants with modification and mobility of PGRs, and products effective in induction of metabolic pathways are represented.

Table 10- Some formulations of plant biostimulants in Iran

Product	Components of the formulation (W/W)	Main crops usage	Rates: Foliar application ($kg.1000L^{-1}$)	Rates: Soil application ($Kg.ha^{-1}$)	Time of application
Botamisol	(45% amino acid)	Fruit trees/citrus/ Olive	2	3	Both products 1-5 days before stress or during stress except for pollination time
Vitalem forte	(13.2% amino acid)	Strawberry	1	1	
		Vitis/ tomato/ potato	2	2	
		Ornamental plants	2	1	
		Cucumber	2	2	
			Foliar application ($L.1000L^{-1}$)	**Soil application ($L.ha^{-1}$)**	
FU TOP	Elements, seaweed extracts Amino acids	Fruit trees, Pistachio	2-3	5	Before anthesis or in the time of fruit set
		wheat, rice	3-4	5	In all the stages of the growth in especially in growth stop
		Citrus, zea maize	2-3	4	
		Tomato, potato, sugure beat	1-2	3	
			Foliar application ($L.1000L^{-1}$)	-	
Drin	Organic N 6.3% Organic C 19% Amino acid 39%	flowers	0.4-0.6	-	First days of vegetative growth
		nursery	0.2-0.3	-	3-4 leaves stage
		Foliage crops	0.5-0.6	-	4-6 leaves stage, replication after 15-20 days
		Tomato, pepper	0.4-0.5	-	After transplanting and during anthesis
		Cucumber, melon	0.7-0.75	-	First days of vegetative growth, 2-3 replication with 15-20 days interval
		Strawberry	0.4-0.7	-	First days of vegetative growth, 2-3 replication with 15-20 days interval
		Tobacco	0.3-0.5	-	First days of vegetative growth, 2-3 replication with 15-20 days interval
		Citrus, apple, olive	0.7-1	-	First days of vegetative growth and after fruit set
		Vitis, olive	0.5-0.7	-	First days of vegetative growth till end of fruit set
		Citrus	0.5-1	-	First days of vegetative growth till end of fruit set

Obviously the scientific researches on this subject are in the primary steps and it is necessary for future research and development of biostimulants as combination of some of the various categories presented in this review.

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