Azadi ka Amrit Mahotsav ISAS Webinar Series: A few Excerpts

Bioistimulants in Agricuture

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ABSTRACT

There is an ever increasing demand for increased productivity and efficient utilization of available resources from the agricultural sector. These challenges, stemming from an ever increasing population, can be addressed through various ways, viz. Identifying better genotypes, Optimizing growth environment and Designing efficient agrochemicals as plant protection agents. Biostimulants are non-fertilizer materials which, when applied to plants in small quantities, have growth promoting/altering effects. They modify plant physiological processes, act inside plant cells to stimulate or inhibit specific enzymes or enzyme systems and help to regulate plant metabolism. They are considered as BEYOND FERTILIZERS AND PESTICIDES. Biostimulants can be used at various stages of plant life cycle: 1. To induce flowering, 2. To spur growth, 3. Early fruit setting, 4. Increase productivity, 5. To improve nutrient assimilation and 6. To improve abiotic stress tolerance.

Biostimulants, based on their Chemical Nature, are classified into 6 groups: Natural (Phytochemicals); 2. Semi-synthetic; 3. Synthetic; 4. Extracts/Processed Extracts; 5. Microbes (Beneficial) and 6. Combination of above. Natural Biostimulants are often mixtures of a variety of compounds with variety of Biological activities and variety of Mechanisms. Many Biostimulants are likely to have multiple functions, along with its Biostimulant activity, such as:. improving availability of nutrients, providing pesticidal effect, and possibly also hormonal effects. Also different Components of Biostimulants can offer different Mode-of-Action. A large number of Biostimulant Components are now used in different Agricultural sectors. Status of Phyto-products (Natural/Semi-synthetic) and Extracts/Processed Extracts used as Biostimulants in Agriculture and related fields are briefly discussed here.

1. INTRODUCTION:

There is an ever increasing demand for increased productivity and efficient utilization of available resources from the agricultural sector. These challenges, stemming from an ever increasing population, can be addressed through various ways, viz. 1. Identifying better genotypes, 2. Optimizing growth environment, 3. Designing efficient agrochemicals as plant protection agents.

Agrochemicals can be broad spectrum, less expensive to produce and easy to implement due to which they hold immense potential over breeding/genetics based approaches. Agrochemicals have their disadvantages (Toxicity to Eco-system) because of which the concept of Organic Farming has arisen. In Organic Farming – "Plant Biostimulants" or simply called as Biostimulants - a promising and envirmental-friendly innovation, plays a major role.

Biostimulants can be used at various stages of plant life cycle:

1. To induce flowering, 2. To spur growth, 3. Early fruit setting, 4. Increase productivity, 6. To improve nutrient assimilation and 6. To improve abiotic stress tolerance.

1.1. Definiation of Biostimulants

From the time since their discovery in 1930, Plant Growth Regulators (PGR, both Stimulants and Retardants) - both natural and synthetic, have seen a steady growth.

The term Biostimulants, were introduced in early 1980s. Zhang and Schmidt defined Biostimulants as "materials that, in minute quantities, promote plant growth". The phrase 'minute quantities' was coined to distinguish them from genral nutrients and other products used for amending soil, the later being used in larger quantities. At this time very few components - such as Seaweed extracts, Alfalfa meal and Willow bark extract - were considered as Biostimulants.

This was followed by good amount of R&D in this area because of which more and more Products were evaluated under field conditions and considered as Biostimulant candidates. By the end of first decade of this century the list became quite big. To incorporate these new "Biostimulants", new definition was proposed in 2012 by the European Commission. According

to this definition: "Plant Biostimulants are substances and materials, with the exception of nutrients and pesticides, which, when applied to plant, seeds or growing substrates in specific formulations, have the capacity to modify physiological processes of plants in a way that provides potential benefits to growth, development and/or stress responses". Here "nutrients and pesticides" were completely excluded. Another definition was proposed by du Jardin in 2015 according to which: "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 nutrient content". This way micro-organisms were included in the "Biostimulants".

Thus Biostimulants are non-fertilizer materials which, when applied to plants in small quantities, have growth promoting/altering effects. They modify plant physiological processes, act inside plant cells to stimulate or inhibit specific enzymes or enzyme systems and help to regulate plant metabolism. They are BEYOND FERTILIZERS AND PESTICIDES.

1.2. Role of Biostimulants in Sustainable Agriculture:

Biostimulants contribute to Sustainable Agriculture by:

1. Improving plant growth (vegetative, reproductive)

2. Improving plant tolerance to abiotic stress on plants, including drought, extreme temperatures and salinity.

3. Enhancing uptake and efficient use of nutrients.

4. Improving soil health by enhancing beneficial soil microorganisms.

5. Enhancing crop quality through plant health and vigor at key stages in the development of crops.

6. Increasing harvestable yields.

7. Indirect effect on Biotic Stress (Pest, Diseases) and
8. Effect on plant nutrition by providing some micro-nutrients.

1.3. Beneficial Effect of Biostimulants:

Biostimulants confer following advantages:

1. Induce germination as well as emergence,

2. Stimulate growth of roots,

3. Promote assimilation of nutrients within plants,

4. Increase tolerance to abiotic stresses and as well as increase water retention in plants,

5. Promotes faster maturity,

6. Increase resistance to disease,

7. Delay seed development triggered senescence

8. Improve crop yields and/or quality.

1.4. Ideal Biostimulant:

An Ideal Biostimulant is:

long acting,

can counters environmental pressures,

has broad spectrum effective on a range of varieties and hybrids,

is easily bioavailable and

has acceptable chemical stability

It should be - Easy to Prepare – Apply –and – Cost Effective.

1.5. Classification of Biostimulant:

Biostimilants: Classified into 4 broad groups.

1. Auxins: Primarily controls growth through cell division and cell enlargement and can be both stimulants as well as retardants (inhibitors). They can cause shoots, buds, and roots. Auxins also act on cell differentiation, aswell as retardants (inhibitors). They cause effect on shoots, buds,

and roots. Auxins also act on cell \ These classes need to be formulated in varying ratios to achieve different objectives, for example, stimulate

2. Gibberellins: Control elongation and division of cells in plant shoots. They act via ribonucleic acid activity stimulation and protein synthesis upregulation in plant cells.

3. Cytokinins: influence cell division, and enlargement, as well as senescence. Also affect transport of amino acids in plants.

4. Ethylenes: As a natural regulator have effect on various cell processes and works in association with auxins.

These classes need to formulated in varying ratios to achieve different objectives, for example, stimulate rooting or to cause early fruting, etc.

Biostimulants, based on their Chemical Nature, are classified into 6 groups:

1. Natural (Phytochemicals); 2. Semi-synthetic; 3. Synthetic; 4. Extracts/Processed Extracts; 5. Microbes (Beneficial) and 6. Combination of above.

2. BISTIMULANT COMPONENTS IN AGRICULTURE

A large number of Biostimulant Components are now used in different Agricultural sectors. Table 1 gives Phyto-products (Natural/Semi-synthetic) and Extracts/Processed Extracts used as Biostimulants in Agriculture and related fields.

Table 1:Biostimulants Used in Agriculture

Phyto-products (Natural/Semi-synthetic)	**Extracts/Processed Extracts**
Abscisic Acid (ABA)	Alfalfa (*Medicago sativa*) Ext.
Acetyl Salicylic Acid (ASA, Aspirin)	*Allium cepa* (Onion) Bulb Aq. Ext.
Adenine sulfate/Adenine Hemisulfate (AdS)	Allium sativum (Garlic) Bulb Aq. Ext.
5 - Aminolevulinic Acid (5-ALA)	*Aloe vera* syn. *A. barbedensis*
6-Benzylaminopyuine (6-BPA)	Apple Cidar Vinegar (ACV)
Brassinosteroids	Bee Honey

Homobrassinoloide	Cinnamon Powder
Caseine hydrolysate	Coconut Water
Cis-Cinnamic Acid (c-CA)	Corn Seed/Maize Grain Ext. (MGE)
Choline chloride (Membrane phospholipids)	*Glycine max* (Soy) Proteins (DSP)
β-Cyclocitral (Prepared from Citral)	*Moringa oleifera* Leaf Water Ext. (MLE)
Follic acid (Vitamin B9)	Mulberry (*Morus alba*) Leaf Water Ext. (MBLE)
Gibberellins (GA)	*Musa paradisica* (Banana) Leaf Ext.
Ploy γ-glutamic acid (γ-PGA)	*Phaseolus vulgaris* (Common bean) Ext.
Glycine (Amino acid)	Seaweed Ext.
Glycine Betaine (GB)	Sorghum (Fodder) Water Ext. (SWE/Sorgaab)
Humic Acid (HA) & Fulvic Acid (FA)	Tea Seed Powder/Saponin-rich waste product
Indole Butyric Acid (IBA)	Vermiwash (VW)
Inocitol/Mayo-Inocitol	*Vigna radiat*a (Mung bean) Ext.
Jasmonic Acid (JA)	Willow (*Salix* spp.) Bark Ext.
N-Acetyl thiazolidine-4-carboxylic acid (NATCA)	*Withania somnifera* Aq. Ext.
Naphthalene Acetic Acid (NAA)	
Phorogluciniol (PG)(1,3,5–Trihydroxybenzene)	
Polyamines (PAs)	
Salicylic Acid	
β-Sitosterol (Phytosterol) (BS)	
Stigmasterol	

Thiourea (TU)	
1-Triacontanol (TRIA)	
t-Zeatin	
Zymostenol	

Let us consider above Biostimulants components in some details with respect to their Mode-of-Action in following 5 categories: effect on: 1. Vegetative growth; 2. Rooting; 3. Reproductive growth; 4. Stress Tolerance and 5. Soil Modification Properties

A. Biostimulants: Phytoproducts (Natural and Semi-synthetic):

1. Abscisic Acid (ABA, Sesquiterpenoid):

1. Vegetative growth - Stimulates the formation and growth of the lateral shoots.

2. Rooting - Stimulates the growth of the root system which includes adventitious roots of the hypocotyl.

3. Reproductive growth - Promotes flowering; plays important role in fruit ripening (strawberry, banana).

4. Stress tolerance - Plays central roles in drought, and high salinity responses, hence generally considered as a stress-related hormone; protect the dormant buds during the cold season, reduces water evaporation by stomatal closure, prevents loss of seed dormancy.

ABA play key role in improving drought tolerance of field crops. Under drought stress condition, ABA synthesis is started in the plant tissues and then it is sent as a stress signal to the stomatal cell. ABA application under drought stress improves drought tolerance by conserving plant cell moisture and improving/maintaining plant growth.

2. Acetyl Salicylic Acid (ASA, Aspirin, Phenyl acid)

1. Vegetative growth - Its application has been found to alter physiological processes of regulation of ion uptake, cell division, cell signaling and stomatal conductance.

2. Rooting - Considered “best rooting hormones” for plant cuttings.

3. Reproductive growth - Induces flowering in crops; has positive effect on fruit appearance of pomegranate fruits.

4. Stress tolerance - Enhanced tolerance to heat, chilling and drought stresses.

3. Adenine Sulfate/Adenine Hemisulfate (AdS, Amine sulfate**)**

1. Vegetative growth - Shoot multiplication enhancement (in plant tissue culture); enhances the growth of isolated meristem tips, induces the proliferation of axillary and promotes the adventitious shoot formation in callus or explants.

2. Reproductive growth - Regulate organogenesis and *in vitro* flowering.

4. 5 - Aminolevulinic Acid (5-ALA, Amino acid)

1. Vegetative growth – Promotes plant biomass accumulation; improves photosynthesis; contributes to plastid-to-nucleus signaling; inhibits ABA-induced stomatal closure; foliar application to plants can increase chlorophyll content in leaves and increases plants' ability to absorb light.

2. Rooting - Promotes seed germination and primary root elongation. Some *in vitro* studies have confirmed the hormonal role of 5-ALA by striking proliferation of callus induction of rooting and shooting with a more effect of the former than the latter.

3. Reproductive growth - improves fruit quality;

- acts as biochemical thinner - thereby thinning fruits by preventing fertilization;

- advances the harvest time of grapes;

- positive effects on fruit weight, fruit color, yield and efficiency index of sour cherry (*Prunus cerasus*) fruits.

3. Stress tolerance - Pre-spraying of ALA enhanced plant tolerance to various abiotic stresses, such as cold and heat stress, water logging, water deficit and salinity stress.

5.6-Benzylaminopyuine (6-BPA, Amine)

1. Vegetative growth - Increases post-harvest life of vegetables by preventing chlorophyll degradation.

2. Reproductive growth - Hormone to induce buds; acts by setting flowers and fruit richness by stimulating cell division; increases in the fruit weight .

Brassinosteroids (BRs, Steroid)

BRs are endogenous plant hormones essential for the proper regulation of multiple physiological processes required for normal plant growth and development. They have a dramatic positive effect on stem elongation.

Under auxin and cytokinin limiting conditions, BRs increase rates of cell division.

BRs also: 1. hastens senescence, 2. hyper-polarize membranes, 3. enhance ATPase activity, and 4. change cortical .They also mediate abiotic and biotic stresses due to salt, drought, temperature extremes and pathogens.

6. Brassinosteroid

1. Vegetative growth - Promotes cell expansion and cell elongation; cell division and cell wall regeneration; promotes vascular differentiation.

2. Rooting - Encourages new root growth.

3. Reproductive growth - Important for pollen elongation for pollen tube formation.

4. Stress tolerance - Can provide protection to plants during abiotic stress.

7. Homo-brassinosteroid

1. Vegetative growth - Promotes cell division and cell elongation; acts synergistically with other endogenous hormones; increases photosynthesis and translocation of assimilation to economic plant parts; increases the levels of enzymes responsible for the synthesis of nucleic acids, proteins and sugars; increases quality of produce.

2. Rooting - Promotes seed germination and increasing early vigor of seedlings.

3. Reproductive growth - Induces flowering, and increasing fruit set and fruit growth.

4. Stress tolerance - Imparts stress resistance under adverse environmental conditions.

8. Caseine hydrolysate (Hydrolysed Milk Protein)

1. Vegetative growth - Plays important role as Biostimulant through the modulation of plant molecular and physiological processes which triggers plant growth; stimulates carbon and nitrogen metabolism and, increases yield and quality of crop.

2. Rooting - Elicited hormone-like activities (Auxin and Gibberellins), promotes root growth; foliar and root applications enhances the uptake and efficiency of both macro- and micro-nutrients; improves nutrient uptake performance of pH-treated plants associated with modifications of root architecture.

3. Reproductive growth - Increases germination.

4. Stress tolerance - Can alleviate the negative effects of abiotic plant stress.

9. Cis-Cinnamic Acid (c-CA, Phenyl acid) (Activity similar to Indole Acetic Acid/IAA)

1. Vegetative growth - Growth-promoting activity (Bio-mass promoter);

- stimulates both cell division and cell expansion in leaves;

- increases biomass.

2. Rooting - Develops larger root system.

Stress tolerance - In rice, β-cyclocitral enhances both root and shoot growth during salt stress.

10. Choline chloride (CC) (Constituent of membrane phospholipids)

1. Vegetative growth - Essential metabolite in plants for growth and development;

- exerts influence on almost every aspect of growth and development;

- exogenous applications has positive effect for greater yield.

1. Stress tolerance - Enables the plant to impart tolerance against abiotic stress due to water.

11.β-Cyclocitral (Prepared from Citral)

1. Rooting - Promotes root stem cell divisions so the root growth and branching is enhanced which makes tomato and rice plant root grow faster; increases primary root and LR growth by inducing cell divisions in root meristems. In rice, β-cyclocitral can also affect root architecture such as the numbers and gravity set-point angle of the roots.

2. Stress tolerance - In rice, β-cyclocitral enhanced both root and shoot growth during salt stress.

12. Follic acid (Vitamin B9)

1. Vegetative growth - Essentially helps plant to grow more heavily and healthily;

- synergistic effects on growth, yield and yield quality of many plant species;

- increases the seed weight, amount of chlorophyll in leaves and synthesis of DNA and RNA;

- has the potential to function as natural antioxidants and growth regulators;

- folic acid treatment increases the productivity of *Pisum sativum*;

- exogenous application of folic acid has positive effect on growth, yield and quality of soybean and strawberry;

- significantly improves growth parameters in potato - plant length, leaf area, chlorophyll, total soluble carbohydrates and total soluble protein.

2. Rooting - Increases the root elongation, germination percentage and stem width at the beginning of germination.

3. Reproductive - Growth (flowering) stimulant; its foliar spray enhances flowering, yield and quality of sweet pepper.

4. Stress tolerance - Folic acid pretreatment reduces the harmful effects of salinity on chromosome aberrances particularly at high salt concentration.

13. Gibberellins (GA, Diterpenoid)

1. Vegetative growth - The most characteristic effects is shoot growth, increased inter-node extension, increased leaf-growth and enhanced apical dominance;

- increased carbon fixation (a secondary effect of increased leaf growth) leads to increased dry weight of plant;

- commercially applied to control the vegetative growth of many crops.

2. Rooting - Treatment with GA does not stimulate growth of intact roots, though some root sections respond by increased growth; stimulates seed germination and also can stimulate root elongation. The seed dormancy is stimulated by gibberellins.

3. Reprodutive growth - Can stimulate flowering and fruit senescence.

4. Stress tolerance - Many forms of dormancy are broken by GA; can increase tolerance to abiotic stresses.

GA stimulates phosphate uptake, potassium uptake, and sulfate translocation from root to shoot in seedlings.

14. Ploy γ-glutamic acid (γ-PGA)

1. Vegetative growth - Can significantly increase the dry weights of shoots in cucumber, wheat productivity, Nitrogen use efficiency, fresh weights of rapeseed and Chinese cabbage.

2. Rooting - Can significantly increase the dry weights of roots, as well as the root to shoot ratio of cucumber seedlings.

3. Soil properties - γ-PGA can affect the Nitrogen turn-over in soil by attracting mineral Nitrogen and enhancing microbial Nitrogen. This would help to temporarily store plant-available Nitrogen, such as fertilizer Nitrogen, when crop demands are small. The stored mineral and microbial Nitrogen would slowly released to crop.

The application of γ-PGA to soil may also affect soil Carbon dynamics.

15. Glycine (Amino acid)

1. Vegetative growth - Growth (flowering and branching) stimulant; shows synergistic effect; plays an important role in vegetative growth; exogenous application can increase Nitrogen status and concentration of mineral elements in plant tissues.

2. Reproductive growth - Shows synergistic effect;

- foliar application of Glycine promotes growth and reproductive growth and the content of essential oil in Jasmine .

3. Stress tolerance - Stress tolerance - It has a chelating effect on micronutrients Iron, Zinc, Manganese, and Copper making their absorption. Soil application of glycine can improve leaf mineral and physiological characteristics which results in higher yield and quality.

16.Glycine Betaine (GB)

1. Preharvest foliar application of GB to banana plant reduces the biochemical and physiological alterations caused by chilling injury.

2. Protects plants from abiotic stresses (drought, heat, cold, and salt stress); it can be applied to leaves or soil to combat dehydration (caused by stress) and prevent crop loss; GB counters stress induced metabolic dysfunctions.

Humic Acid (HA) - Fulvic Acid (FA)

Humic substances are considered as the most important constituents of soils. They form the largest fraction of soil organic matter and play a dominant role in improving soil productivity.

Humic substances are classified as Fulvic Acid with varying pH solubilities with some being soluble at all pH values and some only in alkaline conditions and Humin being insoluble at all pH values.

FAs have the least molecular masses and are the most mobile fraction of humic substances (Molecular weight 250-2500 Da). HAs are soluble under alkaline conditions but are insoluble under acidic conditions. HAs with Molecular weight 50,000 Da are biologically active.

18. Humic Acid (HA) (Considered as a natural rooting hormones)

1. Vegetative growth - Increases cell membrane permeability, oxygen uptake, respiration, photosynthesis and phosphate uptake;

- increases growth, nutrient availability and yield;

- plant fertilization with HA promotes plant growth, development, production and fruit quality of vegetable crops;

- has direct stimulatory effects along with indirect effect on plant metabolism;

- increase fruits and vegetable yields;

- it increases the number of flowers, leaf area and plant height.

2. Rooting - Increases root elongation.

3. Reproductive growth - increases flower yield and quality of gerbera.

4. Stress tolerance - HA improves plant resistance to environmental stresses.

5. Soil properties - Application of HA improves soil aggregation, structure, fertility, and moisture holding capacity. It also increases microbial activity, microbial population, and ion exchange capacity.

HA application - beneficial for nutrient (N, P, K, Mg, Ca, Zn, Fe, and Cu) uptake.

HA stimulates both root and aerial part growth of plants, increase chlorophyll density and may help plant to resist heat and salt stress.

However, when applied to soil, varying fractions of HAs may become insoluble depending upon the soil pH. HAs will also bind to some pesticides reducing their Biological activity.

19. Indole Butyric Acid (IBA) (Auxin synthesized by plants)

1. Vegetative growth - Used on many crops and ornamental plants to promote growth and development and to increase crop yields.

2. Rooting - Mainly used for the induction of adventitious roots (accelerates rooting formation); important ingredient in many commercial horticultural plant rooting products; used on many crops and ornamental plants to promote growth and development of roots.

3. Reproductive growth - Used on crops to stimulate development flowers and the growth of fruits; also used to protect plants during transplantation by stimulating root growth and decreasing shock; used on many crops and ornamental plants to promote growth and development of flowers and fruits; in plant tissue culture IBA is used to initiate root formation in micropropagation.

20. Inocitol/Mayo-Inocitol (Carbocyclic sugar)

1. Vegetative growth - Required for phosphate storage, cell wall biosynthesis, cell-to-cell communication, and storage and transport of plant hormones; play a role in cell division; important for normal plant growth and development.

2. Stress tolerance - Important for the production of stress related molecules.

21.Jasmonic Acid (JA, Sesquiterpenoid)

1. Vegetative growth - Plant hormone - signaling compound involved in the regulation of cellular defense and development in plants;

- JA is directly involved in many physiological processes, including leaf growth, tendril coiling, flower development;

- regulate the production of various metabolites, such as phytoalexins and terpenoids.

2.Rooting - JA is directly involved in root growth; responsible for tuber formation in potatoes and yams.

3. Reproductive growth - has positive effect on flower development;
- Considered for seed treatment to stimulate the natural anti-pest defenses of the plants.

4. Stress tolerance - Stress hormone that regulates plant responses to 1. biotic stresses, such as those elicited by herbivores and pathogens, as well as 2. abiotic stresses such as wounding and ultraviolet radiation.

5. May have a role in Pest Control.

22. **N-Acetyl thiazolidine-4-carboxylic acid (NATCA) (Prepared from amino acid Cysteine and formaldehyde by condensation reaction).**

1. Promotes seed germination and plant cell division.

2. Keeps chlorophyll from being lost.

3. Increases fruit setting rate and fruit yield.

4. Combined with Folic acid as a foliar spray, acts as a "Very Promising Biostimulant".

23. Naphthalene Acetic Acid (NAA)

1.Vegetative growth - Plant hormone – increases stem growth;

- helps to increase cell division and increase overall plant growth;

- increases crop yield and decrease crop drop;

- regulates gender ratio;

- improve blossoms;

- decreases plant ageing and diseases;

- increase grain yield.

2. Rooting - Rooting agent;

- increases root growth;

- finds application in promoting the growth of cuttings, making it particularly useful for breeders and those who are involved in crop transplant.

4. Reproductive growth - Helps fruit growth, increases blossoming, ripening and fruit quality, increases the yield of fruit and decreases the rate at which fruit drops. Can also decrease the amount of seeds in fruit and even produce seedless fruits in some cases.

24.Phorogluciniol (PG)(1,3,5–Trihydroxybenzene) (A precursor in the lignin biosynthesis pathway)

1. Vegetative growth - PG increases shoot formation and somatic embryogenesis in many crops.

- PG has Cytokinin-like and Auxin-like activity; has potential for a range of plant tissue culture studies.

- Useful for maximizing the multiplication rate of woody species which are difficult to propagate.

- PG has also been used to improve the recovery of cryopreserved plants.

2. Rooting - PG exhibits a positive effect on rooting and acclimatization of rooted shoots in micro-propagation of plants;

- PG acts as Auxin synergist.

Exhibits antibiotic effects on *Heliothis armigera*.

25.Polyamines (PAs, Putrocine/Spermidine/Spermine)

1. Vegetative growth – Play an important roles in diverse plant growth and developmental processes.

2. Rooting - PAs regulates in the process of embryogenesis in both angiosperms and gymnosperms.

3. Reproductive growth - Exogenous application of PAs accelerates flower bud differentiation.

4. Stress tolerance - Plays an important roles in environmental stress responses (salt stress, water stress, oxidative stress).

26. Salicylic Acid and other salicylates (SA, Phenolic acid):

Promote root growth in cuttings, seedling, and mature plants of several species.

SA promotes plant photosynthetic rates, production of plant biomass and crop leaf area. It is reported that SA increases the wheat resistance to osmotic stress caused by water deficit conditions.

27. β-Sitosterol (Phytosterol) (BS) (Antioxidant, antimicrobial activities without major toxicity)

Phyto- sterols are precursors in the synthesis of steroid hormones, e.g. Ecdysteroids (insects hormones), and Brassinosteroids (plant hormone).

1. Vegetative growth - a Plant Growth Regulator;

- had a stimulatory effect on growth and yield.

2. Stress tolerance - BS effectively regulates several biological processes to enhance plant resistance against stress factors such as water stress, salt stress, UV radiation stress (abiotic stress) and plant-pathogen interaction stress (biotic stress).

28. Stigmasterol (Phytosterol)

1. Vegetative growth - Foliar application enhances growth and increases productivity of fresh herb and aromatic oil in basil plant;

- a promotive effect on growth, yield and structure of rice plants;

- induces a promotive effect on vegetative growth, anatomical structure of soybean plant;

- increasing stigmasterol concentration can significantly increase sesame growth, seed yield, number of capsules/plant, 1000 seed weight and seed oil percentage;

- Stigmasterol offers promotive effects on lupine plant height and yield;

- offers promotive effect on anatomical structure of stem and leaf of Egyptian lupine.

2.Stress tolerance - Reduces stress; β-sitosterol and stigmasterol accumulate during stress indicating its role as signaling molecules that help in stress adaptation.

29. Thiourea (TU)

1. Vegetative growth - Used to improve plant growth and productivity under normal and stress conditions;

- improves the gas exchange properties;

- improves the root growth and its proliferation;

- exogenously applied, TU improves the sugar metabolism and enhances the proteins biosynthesis. It regulates the plant growth by maintaining higher photosynthetic rate up to the reproductive stage and increases the yield.

2. Rooting - When used as seed pretreatment, TU increases the seed germination; it improves the nutrient acquisition by the root.

3. Stress tolerance - Use of TU is more effective under environmental stress conditions;

- TU is effective in improving plant growth and development. under drought, salinity, heat stress and heavy metal toxicity;

- foliar application of TU is shown to increase the stress tolerance and yield during field trials.

30. 1-Triacontanol (TRIA, Fatty alcohol)

1. Vegetative growth - Stimulant for flowering plants; enhances the physiological efficiency of the cells;

- enhances growth, yield, photosynthesis, nitrogen fixation, enzymes activities, free amino acids, reducing sugars, and soluble proteins of plants;

- enhances plant biomass, chlorophyll, gas exchange parameters, mineral nutrient acquisition, leaf carbonic anhydrase (CA) and nitrate reductase (NR) activity.

- TRIA application increased the plant dry weight, protein, and chlorophyll contents and net photosynthetic rate in rice.

2. Stress tolerance - Plays essential role in alleviating the stress-accrued alterations in crop plants via modulating the activation of the Stress Tolerance Mechanisms.

31. *t*-Zeatin (*t*-Z, Cytokinin isolated from Maize Grain)

The *t*-Z is a natural phytohormone and has the ability to accelerate cell division, induces callus formation, promotes the formation of cotyledons and delays senescence.

1. Vegetative growth - Plant Growth Hormone;

- plays an important role in cell growth, differentiation, induces cell division and stimulates shoot formation.

2. Rooting - Routinely used in plant tissue culture; can also be applied to stimulate seed germination and seedling growth.

3. Stress tolerance- Helps plant to better tolerate environmental stress; has also been shown to promote the resistance of tobacco against the bacterial pathogen *Pseudomonas syringae*.

32. Zymostenol (Cholestrol precursor)

1. Zymostenol is a late-stage precursor in the biosynthesis of cholesterol.

2. It accumulates in cells following administration of microsomal antiestrogen-binding site (AEBS) ligands, such as tamoxifen, which are associated with cell differentiation and a protective type of autophagy.

3. When used alone at a concentration of 20 μM, zymostenol halts the cell cycle at the G0/G1 phase and increases the levels of free sterols, esterified sterols, and triacylglysterols in MCF-7 cells.

B. Biostimulants - Extracts/Processed Extracts

1. Alfalfa (*Medicago sativa*) Extract

1. Vegetative growth - Growth Stimulant used to increase seedling length and dry weight of *Juniperous procera* seedlings.

2. Several crops are known to accumulate dry weight rapidly following applications of small amounts of Alfalfa under greenhouse and growth chamber conditions.

3. Coarsely chopped Alfalfa hay used as band application, increased growth and yield of cucumbers, lettuce, tomatoes, and wheat.

2. *Allium cepa* (Onion) Bulb Aqueous Extract (Anti-bacterial, anti-fungal, anti-oxidant; contains micro-nutrients specially Zinc)

1.Rooting - Can influence the development of root elongation in the cell elongation process in plants; tested on banana root length.

2. Reproductive growth - Foliar application of onion extract on apple (*Malus sylvestris*) trees during dormancy and bud break - flowering percentage and fruit yield characteristics are increased, while number of days recorded to reach full bloom are reduced.

3. Stress tolerance - effect on drought stress of soybean plants: enhanced the tolerance of the plant to drought stress observed.

- high levels of acemannans and saponins provide anti-microbial activities;

3. *Allium sativum* (Garlic) Bulb Aqueous Extract (AGE) (Anti-bacterial, anti-fungal, anti-oxidant)

1. Vegetative and Rooting growth – AGE foliar application to eggplant and pepper seedlings: stimulatory responses in the growth of the vegetables observed.

2. Stress tolerance – AGE foliar application to eggplant and pepper seedlings: resistance against oxidative stress induced by chemical changes observed.

4. *Aloe vera/A. barbadensis*

1. Vegetative growth - Absolutely loaded with nutrients;

- Aloe leaf extract - used to improves vegetative growth of some crop species;

- Aloe leaf powder is used as a Biostimulant to enhance growth and yield of some crop species.

2. Rooting - Rooting substance; *Aloe vera* fertilizer can encourage seed germination and rapid root development, improved cell strength, and contribute to overall superior plant health, growth, and vigor.

3. Stress tolerance - at present many Farmers and Villagers use fresh *Aloe vera* gel for inducing rooting of stem cuttings and air layering of plants.

- contains enzymes and plant hormones that help to reduce transplant shock, and boost the plant's resistance to drought, stress, and disease;

- high levels of acemannans and saponins provide ant--microbial activities;

- this protects plant from pathogenic attack;

- the comulative effect is healthier plants that are more resistant to disease, pest pressure, and abiotic stess.

Considered as a natural rooting hormones.

5. Apple Cidar Vinegar (ACV)

1. Rooting - Can be used as rooting stimulant (used as root treatment); considered as organic rooting hormone.

2. Vegetative growth - Contains phenolic compounds and 30 trace elements;

- has acidic pH; makes soil acidic (healthy for plants);

- has biological activities like anti-oxidant, anti-microbial, anti-fungal and insect control activity;

- can be used as a fertilizer to maintain healthy plants.

6.Bee Honey

Natural growth stimulant.

Considered as a natural rooting hormones.

Several vitamins like Vitamin C and Vitamin B1 present in honey are found to help initiation of roots in cuttings in many plant species.

7.Cinnamon Powder

1. Vegetative growth – Has a stimulating effect for tomato plants.

2. Rooting - Rooting substance.

Antifungal; has potential to inhibit Bortritis *cinerea* growth and also.

Considered as a natural rooting hormones.

8.Coconut Water

- Growth stimulant;

- Cytokines can be extracted and used as Biostimulants.

Considered as a natural rooting hormones.

Coconut water is reported to have many of auxin such as Indole-3- acetic acid, *t*- zeatin and Gibberellins (GA1 and GA3).

9. **Corn Seed Extract/Maize Grain Extract (MGE)**

1. Vegetative growth - MGE is enriched with antioxidants, plant hormones (superoxide dismutase, catalase and peroxidase), vitamins, polyamines, auxins, cytokinins, gibberellins and different macro- and micro-nutrients making it effective Biostimulant.

- MGE - promotes morphological and physio-biochemical processes.

Antifungal; has potential to inhibit *Bortritis cinerea* growth ~~and also~~.

MGE – Evaluated on many crops under Field Conditions with positive effect.

- protects maize from low temperature stress ~~and~~ in wheat from salinity stress.

2. Stress tolerance - Used to prime seeds to enhance plant performance particularly under salinity stress.

- MGE applied to seeds - reported to enhance plant performances under different stress conditions: salinity, draught, nutrient deficiencies and toxicity due to components like cadmium.

MGE – Evaluated on many crops under Field Conditions.

10. ***Glycine max* (Soy) Proteins (DSP)**

1. Vegetative growth - Soybean meal (SBM) - is allowed as a non-synthetic plant or soil amendment;

- addition of DSP - increases the fresh weight of *Brassica rapa*; the growth of *Solanum tuberosum, Solanum lycopersicum*, and *Brassica juncea*.

2. Rooting - DSP (12 mg-peptides/kg-soil) produced thicker roots than a chemical fertilizer; the number of root hairs in *B. rapa* increased and each was elongated when DSP (30 µg/ml) was added.

3. Stress tolerance - Soybean supplies nitrogen into the soil.

11. *Moringa oleifera* Leaf Water Extract (MLE)

1. Vegetative growth - MLE increases plant biomass, dry matter content, branching and yield;

- exhibits Biostimulant activity on many crops.

2. Rooting - MLE - improved seed germination.

3. Reproductive growth - MLE increased flowering, flower retention and increased fruit yield and quality among a wide variety of plant species.

4. Stress tolerance - Effect on chilling stress tolerance in autumn maize;

- protects maize from low temperature stress and wheat from salinity stress.

- MLE contains antioxidants, which prevent, stabilize and terminate the reactions of reactive oxygen species by defending oxidative induced cellular damage. Therefore, external supplementation of anti-oxidants is widely recommended to protect cells from the drought. MLE is a rich source of amino acids, nutrients, auxins, antioxidants, vitamins, and different macro- and micro-nutrients required for plant growth and regulation of enzymes.

- MLE foliar application to crop plants - increased number of roots and plants produce more and larger fruits which ultimately increased 20-35% yield.

- MLE increased soybean (*Glycine max*) yield by 35%.

- MLE improved seed germination, biomass production, and yield of blue panicum grass (*Panicum antidotale*), barnyard grass (*Echinachloa crusgalli*), buffel grass (*Cenchrus ciliaris*), wheat (*Triticum aestivum*), and maize (*Zea mays*). MLE also protected maize from low temperature stress and wheat from salinity stress.

- MLE improved both vegetative and reproductive development of tomato (*Solanum lycopersicum*) plants, including increased fruit yield and quality.

- In agriculture and horticulture, use of MLE has proved beneficial for the growth and yield, deeper root development and better seed germination, delay of fruit senescence, and improved plant vigour and yield quality/quantity. MLE also impart the crops the ability to withstand adverse environmental conditions.

12. Mulberry (*Morus alba*) Leaf Water Extract (MBLE)

1.Species specific plant growth-promotive properties.

2. Application of MBLE promoted the germination and growth of pea (*Pisum sativum*), broad bean (*Vicia faba*)

3. Stimulan activity on wheat crop.

Leaves contains19.4% protein content, anti-oxidants, macro-elements such as Ca, N, K, and Mg with low Na.

13. *Musa paradisica* (Banana) Leaf Extract

1. Contains abundant vegeto-alkali and crude protein.

2. Contains bio-active compounds.

3. Bio-stimulant fertilizer.

4. Can be used as Biostimulant.

14. *Phaseolus vulgaris* (Common bean) Extract

Contains bio-active compounds (Proteins, Minerals, Polyphenols, Auxins).

Can be used as Biostimulant.

15. Seaweed Extracts

1. Vegetative growth - Eco-friendly stimulantes the crop growth and yield; contains growth promoting hormones (Auxins), vitamins, amino acids, antibiotics and micro-nutrients.

2. Rooting - Effect on root development.

3. Reproductive growth - Promotes early flowering, uniform fruit setting and plant productivity.

4. Stress tolerance - Eco-friendly stimulator of crop stress tolerance.

Considered as a natural rooting hormones.

(Commonly used Seaweeds: 1. *Ascophyllum nodosum* (Brown Seaweed); 2. *Gracilaris edulis*; 3. Green microalgae; 4. *Sargassum wightii*; 5. *Spirulina plantensis* (Micro-algae); 6. *Ulva intestinalis* (Green Seaweed).

16. Sorghum (Fodder) Water Extract (SWE/Sorgaab)

1. Vegetative growth - Foliar application - improves photosynthesis, membrane integrity, growth and development.

2. Stress tolerance - Effect on chilling stress tolerance in autumn maize;

- foliar application - affects plant defense system against abiotic stresse;

- seed priming with SWE enhances crop tolerance to salinity stress.

17. Tea (*Camellia sinensis*) Seed Powder/Triterpene Saponin-rich waste product

1. Vegetative growth - Contains high amount of proteins - can be used as fertilizer; pronounced and direct physiological effect on various crops. The effects can be used to enhance crop yield and quality in agriculture and horticulture.

2. Rooting - TPS (dried seed powder-deoiled) improves soil trace elements and promotes the root growth.

TSP - Offers pesticidal and anti-microbial effect, also enhances the resistibility to the pests and diseases;

- TSP is very harmful to earthworms.

18. Vermiwash (VW)

1. Contains several enzymes (protease, amylase, urease and phosphatise), plant growth hormones (IAA, cytokinin, GA3), vitamins, macro- and micro- nutrients, humic acid.

2. VW contains nitrogen fixing bacteria like *Azotobacter* sp., *Agrobacterium* sp., and *Rhizobium* sp., and some phosphate solubilising bacteria.

3. VW serves as pesticide, disease curative and crop tonic and increases the yield of lab beans.
- Rooting stimulant - Tested on banana root length.

4. VW proves to have excellent Bio-pesticidal activities - the plants treated with VW are disease resistant.

5. VW increases soil microorganisms soil improving organic matter decomposition.

6. Vermicompost (VW) and Vermiwash (VC) combination showed significant influence on the biochemical characteristics of the soil.

7. Soil Zn, Mn and Fe content is unaffected with the increasing application of VW.

8. VW is rich in the primary nutrients (N-P-K). Application of VW has been reported to revitalize the soil quality. It rejuvenates the depleted soil fertility and enriches available pool of nutrients, conserves moisture and natural and biological resources.

Considered as a natural rooting hormones.

19. *Vigna radiata* (Mung bean) Extract

1. Contains bio-active compounds.
2. Can be used as Biostimulant.

Willow (*Salix* spp.) Bark Extract (Contains high levels of Salicylic acid/SA and Indole butyric acid/IBA)

1. Vegetative growth - promising effect.
2. Reproductive growth - effects on flowering and callus formation.
3. Stress tolerance - effect on stress mediation.
4. Rooting - Willow water helps cuttings to root.

- Root Promoting Biostimulant (assayed on *Chrysanthemum*); can be utilized to speed-up propagation of soft and semi-hard wood cuttings;

- Approved basic substance with fungicidal properties under agricultural EU pesticide regulation (Regulation (EC) no 1107/2009) since 2015;

- its approved uses are for Argriculture and Viticulture;

- can be used for seed treatment.

A root promoting product "Root Nectar" prepared from Willow bark extract has been found very effective on vegetative cuttings from lavender (soft wood) and chrysanthemum (semi-hard wood). A consistent improvements in the formation of adventitious roots and root branching were achieved. Root Nectar and Willow bark extract can be utilized to up propagation of soft and semi-hard wood cuttings.

20. *Withania somnifera* (Ashwadandha) Aqueous Extract

- Rooting stimulant - Tested on banana root length

- Acts as a potential insect growth regulatory (IGR) - interference with the endocrine system and offers anti-fungal, anti-microbial, anti-oxidant activities.

Banana plantlets with foliar spraying with Ashwagandha Aqueous Extract increased vegetative growth parameters of banana plantlets. The increased growth such as longer plant or higher leaf and root numbers may be due to the highest gibberellin (GA3), auxin (IAA) and cytokinin (zeatin) contained in ashwagandha (11.078, 0.0312 and 0.0149 mg/100g, respectively).

3. CONCLUSION:

Natural Biostimulants are often mixtures of a variety of compounds with variety of Biological activities and variety of Mechanisms. Many Biostimulants are likely to have multiple functions, along with its Biostimulant activity, such as:1. improving availability of nutrients, 2. providing pesticidal effect, and 3. possibly also hormonal effects. Also different Components of Biostimulants can offer different Mode-of-Action.

Challenge is to "Design a Biostimulant Product as per requirements".

Natural Biostimulants Product Development should follow following steps: 1. Selection of Ingredients; 2. Extracts – Standardization (using Markers); 3. Development of Analytical Methods using this Markers; 4. Bio-efficasy; 5. Mode of Action; 6. Composition of Different Ingredients; 7. Compatibility; 8. Toxicity (Phytotoxicity); 9. Safety (Animal); 10. Formulation; 10. Mode of Application (Foliar/Soil/Seed treatment); 11. Shelf Life Studies; 12. Stability Studies; 13. Bio-evaluation (Field Trials); 14. IPR Status (Patenting); 15. Manufacturing and 16. Commercialization. This will lead to New-Novel-Effective-Safe-Cost Effective "Biostimulant Product". New Leads also should be explored by 1. Field observations, 2. Extensive R & D work and 3. Incorporation of Promising Leads into New Biostimulant Products.

Biosketch of Dr. Swati Joshi

Dr. Swati Joshi was formerly the `Senior Scientist' of CSIR-NCL, Pune. During her exciting career of 36 years or so at NCL, she had carried out extensive research in the area of isolation and identification of new-novel molecules, development of botanical pesticides, developement of herbal drugs, neutraceuticals and food additives and had also contributed in the development of processes for obtaining commercially important natural products. She has more than 60 publications in national and international journals including 15 WO Patents. Since last six years, she has been working as Scientific Adviser and Consultant and also as a Evaluator for Funding Agency (BIRAC).