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Role of biostimulants in fruit crops: A review

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Abstract
The effects of fertilizers have an unfavourable effect on algae plants, animals, and people. Due to the fact that man is a higher-order consumer, people are particularly severely exposed to the harmful effects of fertilizer compounds accumulated at the lower levels of the food chain. Harmful compounds from fertilizers may weaken enzymes or interfere with protein production or vitamin absorption in the human body. Natural preparations called biostimulants increase the efficiency of nutrient utilization and tolerance to abiotic stress and improve the quality of crops. Biostimulants include organic and non-organic substances and/or microorganisms. Farmers who manage organic farms are also eager to use natural stimulants to improve crop quality. Increasing consumer awareness concerning healthy food favours the enhancement of the significance of organic farming. The effect of their activities vary depending on the type of biostimulant used and the plant variety. However, it should be noted that most of them have a beneficial effect on crops.

Keywords: biostimulants, fruit crops, algae plants, protein production

Introduction
One of the biggest challenges for agriculture is the development of sustainable and environmental friendly systems to address the need to feed the growing world population. With decreasing area of arable land as we approach the limits of genetic potential of staple crops, the only way to achieve this objective is by increasing the crop yield and protecting what we produce. Simultaneously, quality of crops should be enhanced, particularly under unfavorable growing environments. This means obtaining higher incomes for farmers, having better postharvest storage and more nutritious food for consumers (Povero *et al.*, 2016) [25].
The quality of edible fruits is related to physical (weight, firmness and color), chemical (soluble solids content, titratable acidity and p^H) and nutritional (phenolic content and antioxidant capacity) attributes and, ultimately, depends on consumer security and preferences. Consumer preference is mainly determined by the appearance of the fruit, which is linked to visual aspects such as shape, size, color uniformity, damage marks, maturity degree and weight. Although it is not a confirmation of internal quality, the preservation of visual quality has been the main target of post-harvest technology. However, this view is changing because of the growing concern of consumers about the nutritional benefits of fruit consumption and the demand for―functional foods. It should be noted that quality attributes may vary according to species. For premium grape used to produce red wine, the phenolic content and composition are important attributes as they are directly related to wine quality.
One of the important attributes for apple consumers is the red color, which is determined by the concentration of anthocyanins. The quality of the fruit that reaches the consumer depends on agronomic practices, environmental conditions and post-harvest technology. Since, the appearance and nutritional quality are fundamental for the acceptance of fruit by the consumer, there is a great search for the adoption of new techniques and agricultural inputs to meet this demand. At the same time, farmers seek ways to increase productivity in a sustainable manner, to guarantee security and food quality for the consumer, which in return may represent good profits. Towards these goals, the use of plant biostimulants is among the emerging techniques.
Regarding fruit quality, much less has been done, but the available results indicate that plant biostimulants can improve appearance, size and sometimes shape, and sensory characteristics.
The quality and quantity of crops are influenced by both biotic and abiotic factors. Quality may be defined as a set of agronomic (e.g., fruit size, yield, resistance to bacteria and fungi) and organoleptic (e.g., colour, shape, firmness) properties as well as nutrient and vitamin content.

The abiotic factors include soil composition, extreme salinity, acidity, high and low temperatures, drought, pollution, humidity, rain, wind, or ultraviolet radiation. Stress caused by unfavourable stimuli can significantly reduce harvest yields because plants respond by using their energy reserves to fight stress instead of concentrating on yielding. Biotic factors include various bacteria, fungi, or viruses that are the cause of numerous plant diseases. Fungal and bacterial infections may not only reduce yield but may also lead to the loss of the entire harvest. To prevent this, various types of plant protection products are used. In accordance chemical and mineral plant protection agents are intended to be slowly replaced by natural preparations. The reason for this is the adverse influence of chemical and mineral plant protection agents on the natural environment, as well as on the health benefits of plant crops. Moreover, artificial fertilizers are responsible for the eutrophication of many bodies of water. This results in the formation of dead zones devoid of living organisms.

Definitions and Classification of Biostimulants

Biostimulants can be treated as an additive to fertilizers and support the uptake of nutrients, promote plant growth, and increase tolerance to abiotic stress. The definition of biostimulants is wide and not sufficiently precise. However, there are two main features that distinguish biostimulants from other growth and plant-protection agents. A biostimulant may be any substance or mixture of substances of natural origin or microorganism which improves the condition of crops without causing adverse side effects. Enzymes, proteins, amino acids, micronutrients, and other compounds may be used as biostimulants. Natural stimulants are often included under the term biostimulants, including phenols, salicylic acid, humic and fulvic acids, or protein hydrolases.

An important group of plant biostimulants are organisms including fungi and bacteria that change the species composition of organisms found in the soil or plants. Their presence may accelerate the rate of degradation processes or limit the number of specific fungal and bacterial groups. Popular fungi used as biostimulants include *Glomus intraradices*, *Trichoderma atroviride*, *Trichoderma reesei*, and *Heteroconium chaetospira*. Useful bacteria include *Arthrobacter spp.*, *Enterobacter spp.*, *Acinetobacter spp.*, *Pseudomonas spp.*, *Ochrobactrum spp.*, *Bacillus spp.*, and *Rhodococcus spp*. Biostimulants cannot be defined as fertilizers because they do not provide nutrients directly to plants. Biostimulants may facilitate the acquisition of nutrients by supporting metabolic processes in the soil and plants.

1. Humic acid

- Collection of natural components of the soil organic matter with relatively low molecular mass that result from the decomposition of plant, animal and soil microbes, which can be split into three main categories: humic acid (HA), fulvic acid (FA) and humin.
- Most sources of humic substances used in horticulture are non-renewable and include natural humidified organic matter, such as peat and organic soils and mineral deposits.
- More sustainable, renewable sources are derived from compost and vermicompost.

Benefits of Humic substances v Chemical Benefits

- Humic substances serves as a buffer to neutralize both excessive soil acidity and alkalinity.
- Induces high CEC ensuring retention of nutrients by roots for later assimilation in plant.
- Improves both the uptake and retention of vital nutrients.

Physical Benefits

- Make soils more friable or crumby-breaks up hard pan (dense layer of soil).
- Increases water holding capacity (up to 4 times).
- Increases aeration of soil.
- Reduces soil erosion.
- The darker color imparted leads to greater absorption of solar energy providing warmer sub soil temperature.

Biological benefits

- Increases germination of seed.
- Stimulates root development.
- Various growth regulators, vitamins, amino acids, auxins and gibberellins are formed as organic matter decays and enhances growth.
- Feeds microorganisms that recycle nutrients and produce antibiotics.

2. Seaweed extracts

- Seaweeds are brown, red and green marine macro algae.
- The most commonly used seaweeds in horticulture are the brown seaweeds, including species of the genera *Ascophyllum*, *Fucus* and *Laminaria*.
- The biological activity of these extracts depends strongly on the raw material and extraction process, which could be alkali extraction, acid extraction or any other technology.
- Major components–polysaccharides (30-40%) of the dry weight and include alginates and laminarins.
- Polysaccharides possess plant growth promoting activities and are known to elicit plant defence responses against fungal and bacterial pathogens.

Table 1: Effect of seaweed extracts on fruit crops

Crop	Seaweed extract	Effects	References
Apple	A commercial extract of *A. nodosum*	Reduced alternate bearing	Spinelli *et al.* (2009) [34]
Banana	*Ochtodes secundiramea* & *Laurencia dendroidea*	Inhibited anthracnose	Machado *et al.* (2014) [18, 19]
Grape	*A. nodosum* commercial extract	Increased in Cu uptake, K^+ and Ca^{2+}, Increased in berry size, weight and firmness, yield, uniform ripening	Turan and Kose (2004) [36]; Mancuso *et al.* (2006) [20]
Orange	*A. nodosum* Seaweed extract	Increased in growth under drought stress, Increased in fruit weight, quality, total soluble solids and sugar	Spann and Little (2011) [33]; Kamel (2014) [16]
Papaya	*Ochtodes secundiramea* & *Laurencia dendroidea*	Inhibited anthracnose	Machado *et al.* (2014) [18, 19]

3. Chitosan

- Chitosan is deacetylated form of chitin, a naturally occurring component of fungal cell walls, nematode egg shells and the exoskeleton of insects and crustaceans.
- Chitosan is produced naturally as well as industrially.
- The physiological effects of chitosan are oligomers in plants i.e. it results in binding of this polycationic compound to a wide range of cellular components, including DNA, plasma membrane and cell wall constituents and also helps to bind the specific receptors involved in defense gene activation.

Chitosan as biostimulant helps in

- Protection against fungus, bacteria and virus.
- Tolerance to abiotic stress (drought, salinity and cold stress).
- Enhancement of quality traits related to primary and secondary metabolisms.

Application

- Seed-coating agents, foliar treatments and postharvest coatings of fruits to prevent postharvest decay and increase the shelf life.

Modes of action

Affects cell membranes, alters DNA and activates defense genes.

Table 2: Response of fruit crops to chitosan application

Crop	Chitosan type and concentration	Method of application	Effects	References
Mango	Irradiated crab shell chitosan	Fruit coating	Extend the shelf life Maintain eating quality up to four weeks	Abbasi *et al.* (2009) [1]
Banana	Chitosan at 1% in 0.5% acetic acid	Hand dipping in post harvest	Delayed ripening (changes in peel color, firmness and soluble solids)	Win *et al.* (2007) [37]
Citrus	Chitosan at 0.1% and 0.2% in acetic acid	Fruit dipping	Inhibited *Penicillium digitatum* Improved firmness, acidity and ascorbic acid	Chien *et al.* (2007) [9]
Papaya	Chitosan at 1% in 10% acetic acid	Fruit coating	Decreased respiration Reduced disease incidence and severity on inoculated fresh papaya	Hewajulige *et al.* (2009) [15]

4. Protein Hydrolysates and other N-containing compounds

- Protein hydrolysates (PHs) are an important group of plant biostimulants are mixture of peptides and amino acids, mainly produced by enzymatic or chemical hydrolysis of proteins from animal or plant derived raw materials or agro industrial by-products.

PHs are known to

- Increase microbial biomass and activity.
- Soil respiration and soil fertility.
- Nutrients availability and acquisition by roots (Chelating and complexing activities of specific AAs and peptides).
- Tolerance to abiotic stress.

Modes of Action

- Protein hydrolysates can increase soil fertility and soil microbial activities, thereby indirectly improving plant growth and productivity.
- Direct effects of these materials include hormonal activities and ensuring primary and secondary metabolism through regulation of genes and enzymes involved in N assimilation and TCA (tricarboxylic acid) cycle.
- Some amino acids have chelating properties and can serve as protectants against heavy metal induced stress, while others contribute to micronutrient acquisition and mobility.

Table 3: Effects of protein hydrolysates (PHs) on horticultural crops

Crop	Type of PH	Application mode	Experimental conditions	Effects	References
Banana	Chicken feather derived PH	Root and foliar	Field trial	Early flowering; increased nutrient, chlorophyll content in leaves; reduced sugars, proteins, amino acids, phenolics and flavonoids in fruits	Gurav and Jadhav (2013) [14]
Kiwifruit	Animal derived PHs with different molecular weights	Foliar	Pot trial	Shoot and root biomass were increased by PH fractions with the lowest molecular weight especially at low rates	Quartieri *et al.* (2002) [27]
Passion fruit	Animal derived PH	Foliar	Nursery	Increased seedling growth	Morales- Pajan and Stall (2004) [23]
Grapevine	PH of distiller‘s dried grains and carob germ flour PHs from soybean or casein	Root and Foliar	Field trial	Increased total phenolics and anthocyanin in grape juice. Up-regulated defense genes increased resistance to *Plasmopara viticola*	Boselli *et al.* (2015) [39]

5. Silicon

- Silicon is a biostimulant in the group of inorganic products.
- Important beneficial element found most abundant on the earth.
- In the soil solution, silicon occurs as non-ionic silicic

acid, which is easily taken up by plant roots and moved throughout the plant.

- Generally two types are found: solid and liquid form.
- It is mainly deposited at the end points of the transpiration stream in cell walls, cell lumens and intercellular spaces in the form of hydrated amorphous silica.

- **Effects of silicon**
- Promote plant growth.
- Enhance quality of plant products.
- Modulate nutrient and water mobility.
- Tolerance to abiotic stress.
- Other stress-alleviating effects of silicon include its ability to immobilize toxic metals in plant tissues & soil and to delay plant senescence processes.
- Protection against heavy metals toxicity.
- Increase plant hormone synthesis and signaling.

Beneficial fungi

Beneficial fungi with plant biostimulant activity are found in group of symbiotic fungi, particularly Arbuscular Mycorrhizal Fungi (AMF) within the genus *Glomus*, which penetrate plant roots and form a highly branched tree like network of roots and hyphae This network enables the plants to extend their root system beyond the depletion zone, allowing for enhanced uptake of nutrients and water and rendering them more tolerant to drought stress AMF is their susceptibility to different crop management practices, such as soil tillage, bare fallow periods and high levels of fertilizers.

Fungal-based products applied to plants

- As a biofertilizers.
- Enhance nutrient use efficiency (phosphorous deficient soil).
- Maintain phytohormone balance.
- Helps in organ growth and morphogenesis.
- Improve crop yield and product quality.
- Induce changes in secondary metabolism leading to improved nutraceutical compounds.

Table 4: Effects of inoculation with AMF on the agronomical, physiological and biochemical performance of horticultural crops under drought conditions

Horticultural species	Mycorrhizal species	Growing conditions	Crop performance and stress tolerance	Reference
Trifoliate orange	*Glomus versiforme*	Greenhouse	Inoculation increased fresh, dry weight and leaf area of seedlings under drought stress due to improved uptake of P, K and Ca	Wu and Zou (2009) [26]
Pistachio nut	*Funneliformis mosseae* and *Rhizophagus intraradices*	Greenhouse	Inoculated pistachio plants had higher P, K, Zn and Mn leaf concentrations than non inoculated plants	Bagheri *et al.* (2018) [3]
Strawberry	*F. mosseae, F. geosporus* and mixed inoculation	Greenhouse	Inoculation with one or two fungal species increased strawberry growth, yield and water use efficiency (WUE) under water stress conditions	Boyer *et al.* (2015) [8]

6. Beneficial bacteria

- With regard to the horticultural uses of biostimulants, that promote plant growth, known as PGPBs (plant growth-promoting bacteria), includes- Free-living bacteria that inhabit the zone around the root (ectorhizosphere), bacteria that colonize the root surface (rhizoplane) and bacteria that live within the roots (endorhizosphere).
- Bacteria with plant growth promoting activity are found in the genera *Bacillus, Rhizobium, Pseudomonas, Azospirillum, Azotobacter* and many others.
- One of the best effects of PGPBs on plants is their ability to fix nitrogen, also to produce siderophores, small iron-chelating compounds that reduce the growth of deleterious soil borne pathogens.

PGPR inoculants are now regarded as plant 'probiotics'

- Supply of nutrients.
- Increase in nutrient use efficiency.
- Induction of disease resistance-Volatile organic compounds (VOCs) such as 2,3butanediol (2,3-BD).
- Enhancement of abiotic stress tolerance.

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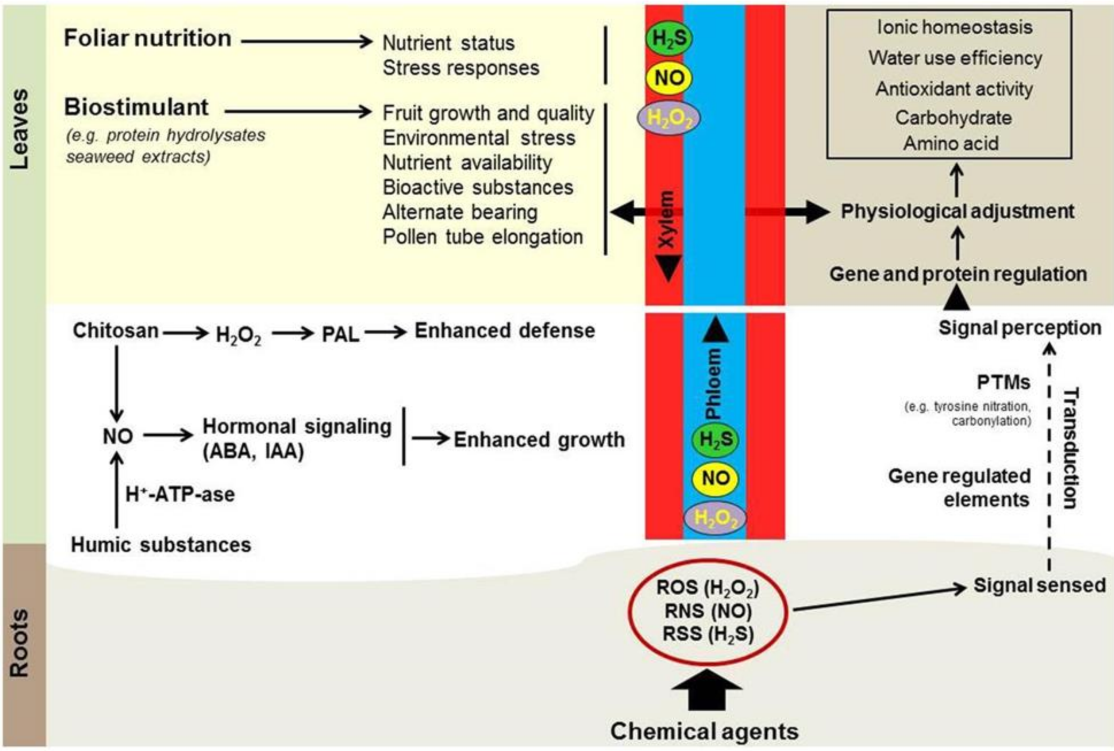

Figure 1| Model of likely biostimulant interaction with H_2O_2, NO, and H_2S priming signaling in fruit trees. (Georgia *et al.*, 2017) [13]

Sindha *et al*. (2018) [32] found that the application of humic acid at 1% was most effective treatment and recorded significantly maximum fruit yield/plant, fruit weight, fruit diameter, total soluble solids, total sugar, reducing sugar, non-reducing sugar, ascorbic acid shelf life of custard apple (*Annona squamosa* L.) cv. local. Humic acid is one of the bio-stimulants which promote plant growth (higher biomass production) by accelerating cell division.

Barbosa *et al.* (2016) [4] conducted an experiment on the effects of fertigation with humic substances, with and without the addition of plant extracts, on the root system of the 'BRS Princesa' banana cultivar. The experimental design was randomized blocks, in a split-split-plot scheme with five replicates, for the following factors: humic substance doses and presence of a plant extract formulation. The treatments consisted of five doses of humic substances, based on a reference dose of 14.14 L ha^{-1} $cycle^{-1}$, in the presence and absence of plant extract. The variable root length (cm) was subjected to variance analyses to evaluate the effect of the humic substances, either isolated or in interaction with plant extract and soil depth. The use of the plant extract increased the effect of the humic substance on root length but overshadowed its effect for the different doses of humic substance. Root length was not sensitive to increasing humic substance dose with plant extract for doses of up to 42.42 L ha^{-1}at 0-0.40 m depth.

Patel *et al.* (2020) [24] conducted a study with treatments comprised foliar application (At 50% flower opening stage, pea stage and marble stage) of of humic acid @ 1, 1.5 and 2%, salicylic acid @ 1000, 1500 and 2000 mg/l and novel liquid @ 1, 1.5 and 2%. Among all the treatments T_2 (Humic acid @ 1.5%) treatment was found most effective treatment and recorded significantly maximum shelf life, TSS, total sugar, reducing sugar, non reducing sugar and ascorbic acid and minimize the acidity, PLW and spoilage in mango cv. Amrapali.

In a study conducted by Sau *et al.* (2015) [31] was observed that the application of plant biostimulants significantly improved plant growth, fruit set, and yield as well as improved fruit physico-chemical properties and it was concluded that spraying of Biozyme @ 10 ppm to the rainy season guava cv. Allahabad Safeda, one at flowering and second after one month of first spray not only gave more yield to the farmers but also improve the fruit quality.

Dash *et al.* (2021) [12] investigated in mango that foliar application of *Ascophyllum nodosum* extract @ 3000-5000 ppm thrice at panicle initiation, pea, and marble stages of fruit growth was effective for reducing fruit drop and enhancing quantum and quality of produce. 3000 and 5000 ppm *Ascophyllum nodosum* extract treatments outperformed all the treatments of triacontanol. Plants sprayed with 5000 ppm *Ascophyllum nodosum* extract recorded the maximum value for fruit retention (48.15, 36.74, 29.48, and 25.76% at 3, 6, 9, and 12 weeks after pea stage, respectively), number of fruits (133.25 per tree), fruit weight (188.84 g per fruit), and yield (25.16 kg per tree), whereas the lowest values were recorded under control. With respect to fruit quality, 3000 ppm ANE produced the fruits with the highest TSS (19.62 °B), whereas the highest TSS/acid ratio was recorded with 5000 ppm ANE (62.29) treatment.

Ravi *et al*. (2018) [28] evaluated the effect of different seaweed bio-formulations developed by Sea6 Energy Pvt. Ltd. Bangalore, on growth, yield and quality of Banana cv. Grand Naine. Four different seaweed formulations (LBS) were applied as foliar spray at vegetative and early flowering stages of the plant. It was observed that foliar application of LBS6S@ 1ml/L improved the bunch weight significantly by 25.24% over control followed by LBS3@ 5 ml/L with 12.62% over water control. The number of hands and fingers per bunch also increased to 5.78% and 6.6% respectively with LBS6S @ 1 ml/l treatment over control.

Krishna and Rao (2014) [17] studied the effect of chitosan coating on the physico-chemical characteristics of guava cv. Allahabad Safeda fruits stored at room temperature (28-32 °C and 3241% RH) was investigated. The fruits were treated with chitosan (1% and 2%) to study its effect on the postharvest life and quality of guava. Various storage attributes were studied at successive intervals of storage. In general, Physiological Loss in Weight (PLW %), respiration rate and TSS increased while firmness and acidity decreased with storage duration. Among various treatments, 1% chitosan treatment was found effective in extending the shelf life upto7 days, as it delayed ripening and retained greater firmness with reduced PLW%. However, the fruits treated with 2% chitosan showed uneven ripening and did not turn yellow even at the end of shelf life, attributed to CO_2 injury.

Bhavana *et al*. (2019) [6] conducted studies on the effect of surface coatings and packaging materials on quality of Apple ber (*Zizyphus mauritiana*) at different storage conditions. The experiment was conducted in two factor in which factor one includes nine treatments of surface coatings and packaging materials along with control (without any surface coating and packaging). Factor two includes two storage condition S_1-cold storage (10±2 ºC) and S_2-room temperature (22±2 ºC) and observations were recorded at 3 days interval upto end of shelf life of fruits. Among the treatments Chitosan 1% + HDPE (High Density Polyethylene) is best followed by Chitosan 1% + PP (Polypropylene). Among both storage conditions fruits stored in cold storage (10±2 ºC) gave better results. In interaction effects Chitosan 1% + HDPE (High Density Polyethylene) + cold storage (10±2 ºC) is best of all the treatments followed by Chitosan 1% + PP (Polypropylene) + cold storage**.**

Ashraf *et al.* (2013) [2] conducted a field study in which pecan trees under investigation were subjected to foliar spray of 0.5% urea, 0.1% boric acid, 0.5%, zinc sulphate, 5 ml/L supramino and their combinations. The results revealed that the foliar application of 0.5% urea, 0.1% boric acid, 0.5%, zinc sulphate and 5 ml/L supramino resulted in better nut quality in comparison to control. It was found that leaf nutrient contents (N, P, K, Ca and Mg) were also recorded maximum in trees treated with 0.5% urea+0.1% boric acid+0.5% zinc sulphate+5 ml/L supramino. Maximum leaf iron content was recorded in trees treated with 0.5% urea and 5 ml/L supramino whereas, trees sprayed with 0.5% urea, 0.1% boric acid, 0.5% zinc sulphate and 5 ml/L supramino was found to have highest leaf Zn, Mn and Cu contents.

Das *et al*. (2017) [11] studied the effect of Silica on physical and biochemical characters of guava, they used diatomaceous earth is a source of silicon it was applied after bahar treatment as whole basal application along with RDF. RDF + 3 kg/plant of DE showed best results with respect to fruit weight, fruit volume, fruit girth, TSS, Total sugars and Ascorbic acid content.

Barman *et al*. (2007) [40] investigated the influence of AM fungi on growth and grafting success of Rangpur lime by inoculating the AM fungi consisted of a mixture of sand, soil and FYM in (1:1:1) (v/v) proportion and root segments of maize and ragi comprising of hyphae, arbuscles, vesicles and chlamydospores of AM fungi. The inoculation was done at five grams per polybag. They found that *Glomus mosseae* inoculated plants showed better graft success and survival percentage

Conclusions and Future Prospects

- The biostimulants can partly substitute the chemical inputs and restore soil fertility particularly by fostering the development of complementary soil microorganisms which facilitate enrichment of nutrients in soil.
- In plant, it not only enhances the quality traits but also helps in tolerance to biotic and abiotic stress, enhances the nutrient efficiency, increases yield and gives long term benefit in a eco-friendly way.
- During their life cycle, crops are often exposed to abiotic stresses, acting individually or in combination, which could dramatically reduce the yield and quality of products. Biostimulants could represent an effective and sustainable tool to enhance plant growth and productiveness, improving tolerance against abiotic stresses. In fact, biostimulants have been successfully applied for:
- Improving nutrients and water use efficiency of crops;
- Enhancing tolerance against salinity, water stress, cold, high temperature, etc.;
- Increasing yield and quality of agricultural crops.

It is important to consider that the complex and variable nature of raw materials used for their production and the heterogeneous mixture of components of the final product can make it difficult to attribute a specific mode of action to each biostimulant. The situation is further complicated by the high number of plants, bacteria and in general, substances included into the category of plant biostimulants. For example, two products obtained by two different plants would fall in the same category, but their effects and their mode of action might be completely different. Moreover, the opposite situation may occur; the same product may produce different effects when applied on different plants. This could be related to the genetic variability among species, variety or cultivars. In addition, the biostimulant activity of a product may also depend on the nature and severity of the abiotic stress. It must also be considered that trying to link a specific mode of action only to the main component of a product might be a mistake because it would be like excluding the effect of the molecules that are presents in small quantities or in traces, but it is known that the efficacy of biostimulant products is the result of a synergistic or antagonistic effect of many components (Roberta *et al*., 2019) [29].

Furthermore, our understanding of the mode of action also depends on the amount of information provided by scientific papers, on the numbers of analyses performed, and on their investigation level. The availability of innovative research tools will surely improve the knowledge of biostimulant composition, but this information will not be exhaustive. Therefore, the biostimulant mode of action can be understood through plant responses at the physiological, biochemical, and molecular levels.

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