The Pharma Innovation Journal 2022; 11(10): 1283-1288

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(10): 1283-1288

www.thepharmajournal.com
Received: 20-07-2022
Accepted: 28-08-2022

Manonmani S
Department of Horticulture, Central University of Tamil Nadu, Thiruvarur, Tamil Nadu, India

Senthilkumar S
Department of Horticulture, Central University of Tamil Nadu, Thiruvarur, Tamil Nadu, India

Manivannan S
Department of Horticulture, Central University of Tamil Nadu, Thiruvarur, Tamil Nadu, India

Corresponding Author:
Senthilkumar S
Department of Horticulture, Central University of Tamil Nadu, Thiruvarur, Tamil Nadu, India

Multilevel functionality of biostimulants in sustainable horticulture for modern era

Manonmani S, Senthilkumar S and Manivannan S

Abstract
A plant biostimulant is any substance or microorganism applied to crop or soils with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits regardless of its nutrients content. Major categories of plant bio-stimulants are humic and fulvic acids, protein hydrosylates, seaweed extracts, botanicals, chitosan and other biopolymers, inorganic compounds and beneficial micro-organism. Humic acid increases the antioxidation capacity under several abiotic stresses and up regulates the biosynthesis of defense-related secondary metabolites. Drenching of humic acid (300 ppm) in nutrient solution could be used as a suitable strategy to maintain growth and yield of strawberry under salinity. (Saidimoradi, *et al*., 2019). Protein hydrolysate stimulates phenylalanine ammonia lyase (PAL) enzyme and production of flavonoids under salt stress. Seaweeds can be used as source of organic matter and as fertilizer. They improve mineral composition of plant tissues especially micro nutrient *viz*., Fe, Zn, Mg, Cu. Similarly, NSKE a plant based extract reduce *T. semipenetrans* population by 56.8–58.1% in soil and 46.4–56.2% in root. (Nagachandrabose, *et al*., 2021). Microbial biostimulant-induced plant growth enhancement through the improvement of biological N2 fixation, solubilization of minerals and other nutrients and increasing plant access to soil nutrients help to reduce the yield gap under adverse environmental conditions. PGPR increases the root foraging capacity and enhance the nutrient use efficiency when they applied to plants. Likewise, foliar application of humic acid @ 60 ml/L during bud burst stage and soil application of bio-inoculants (PSB and A. chroococcum @ 10 ml/plant) along with 80% RDF of NPK increase the vegetative growth traits, flowering, fruit yield and quality. (Ashwini, *et al*., 2022).

Keywords: Biostimulant, fulvic acid, humic acid, PGPR and Protein hydrolysate, seaweed extract

Introduction
A plant biostimulant are biologically derived products most widely used to upgrade the production and productivity of crops. A great demand in the market for biostimulant is created in recent times either directly by the farmers as they are eco-friendly and cost effective in nature or indirectly by the consumers of organic produce where biostimulants have proven to be an effective alternative for the synthetic agricultural inputs. Biostimulants are able to perform both the actions of fertilizer and agrochemicals but they are distinguishable from each other. Biostimulants are not a fertilizer because they have no direct effect on increase of plant growth and productivity rather, they improve the productivity by enhancing the efficiency of nutrient uptake of already existing nutrient in soil or externally applied nutrient. And also biostimulants are different from agrochemical as biostimulants take no action against pest and disease where biostimulant induce resistance in plants inorder to reduce pest and disease in field. Any substance or microorganism applied to crops or soils with the intention of increasing plant nutrient efficiency, tolerance to abiotic stress, and/or crop quality traits despite its nutrient status is referred to as a plant biostimulant.

Major categories of plant bio-stimulants
Bio-stimulants are derived from organic sources and are assorted into various category *viz*., Humic substances (such as humic and fulvic acids), protein hydrosylates, seaweed extracts, chitosan, botanicals and other biopolymers, inorganic compounds and beneficial microorganism.

1. **Humic substances** are the substance of natural origin formed as a byproducts of the process decomposition of plant, animal and microbial residues. And also formed from the soil microbial activity by utilizing those substrates. Humic substances are huddle of heterogeneous compounds and are classified into humins, humic acids and fulvic acidsbased on the molecular weight and their miscibility.

2. **Amino-acids and peptides mixtures:** Are the compounds produced as byproducts of agro industries of both plant and animal wastes such as residues of plants, collagen, epithelial tissues by the chemical and enzymatic protein hydrolysis process.

3. **Seaweed extract:** Obtain from different algae which act as source of organic manure. Though the usage of seaweeds in agriculture werefamiliar to mankind, their potential of being biostimulants are recognized only in recent times.

4. **Microorganism based Biostimulants:** Includes beneficial bacteria, chiefly PGPR, and beneficial fungi namely arbuscular mycorrhizal fungi (AMF). They are endosymbiotic or rhizospheric free-living in nature.

Table 1: Use of Bio-stimulants

Humic acid	Improves the nutritional status of leaves especially N, P, K and Ca and makes plants healthy Correct iron chlorosis in leaves
Fulvic acids	Enhance the root density and fruit yield. Resulted in higher dry matter production and increase potassium level in fruits.
Leonardite (an oxidized lignite with high content of humic acid)	Increase the concentration of all nutrients in leaves except Mg and enhance the quality of fruit, particularly on chemical properties such as pH, TSS, total phenolic content, and antioxidant activity.
Glycine betaine	Positive effects in improving soluble solids, vitamin C, polyphenols, and antioxidant activity. Reduce acidity level of fruits and produce bigger fruits.
Seaweed extract (*Ascophyllum nodosum*)	Predominantly increase the number of fruits, fruit weight results in higher production yield. Enhance the quality of fruits by increasing the soluble sugars, organic acid, and carotenoids content in fruits. Reduce fruit cracking up to 10% Positive effects on leaves by lowering the cell membranes damages and increase leaf moisture level.
Inorganic Compounds	
(potassium silicate)	Increased fruit flesh firmness
Beneficial bacteria (*Trichoderma harzianum*)	Enhance the plant growth and development through alteration in plant hormonal content
Beneficial fungi (mixture of AMF)	Impact on characteristics of roots i.e increases the root volume, fresh and dry weight of roots. Higher Zn and P content

(Afonso, *et al*., 2022) [1]

Effects of biostimulants at Cellular level

- Under stress condition, humic acid increase the antioxidation capacity and upregulates the biosynthesis of secondary metabolites which are related to defense mechanism of plant. (Jindo *et al*., 2020) [15]
- *Ascophyllum nodosum* extracts trigger the genes which are responsible for the encoding transporters of micronutrients particularly Cu, Fe, Zn in *Brassica napus*. (Billard *et al*., 2014) [3].
- Enzymatic hydrolysates obtained from Medicaco sativa (alfalfa) triggers the phenylalanine ammonia lyase (PAL) enzyme and regulate gene expression (Ertani *et al*., 2013).
- *Azospirillum brasilense* liberate auxins and its initiates signalling pathways associated with root morphogenesis of winter wheat (*Triticum aestivum*) (Dobbelaere *et al*., 1999) [5]

Physiological function of biostimulant

- Humic acid tend to increase the growth of the linear roots` and increase the biomass of root (Jindo *et al*., 2020) [15]
- Seaweed extract: increase the tissue concentrations and aids in nutrient transport from root to shoot mainly the micronutrient in plants
- PGPR increases the density of lateral root and extends the surface area of root hairs
- Protein hydrolysate has defensive mechanism against UV and oxidative damage through the production of flavonoids during stress condition mainly salt stress. (Huang *et al*., 2010) [16]

Horticultural function of biostimulant

- Humic acid and PGPR both able to increase nutrient absorbing capacity and improve the nutrient use efficiency
- Protein hydrolysate application resulted increased abiotic stress tolerance in crop
- Seaweed Extract improve nutrient status of plants

Economical/environmental benefits of biostimulant

- High yield with minimum fertilizers and diminished the environment losses can be achieved through the use of humic acids and PGPR
- Seaweed extract increase the nutrient contents (S, Fe, Zn, Mg, Cu) in plant tissues and there by seaweed act as agent for biofortification of nutrients in plant cells
- Superior yield even during stress conditions (e.g. high salinity) made possible through application of protein hydrolysate

Mechanism of actions of biostimulants

A clear mode of action and the mechanism of biostimulants on plants are studied in detail till date. However there the several assumptions and hypnotized theories were proposed for the mechanism of actions of plant biostimulant which are illustrated below.

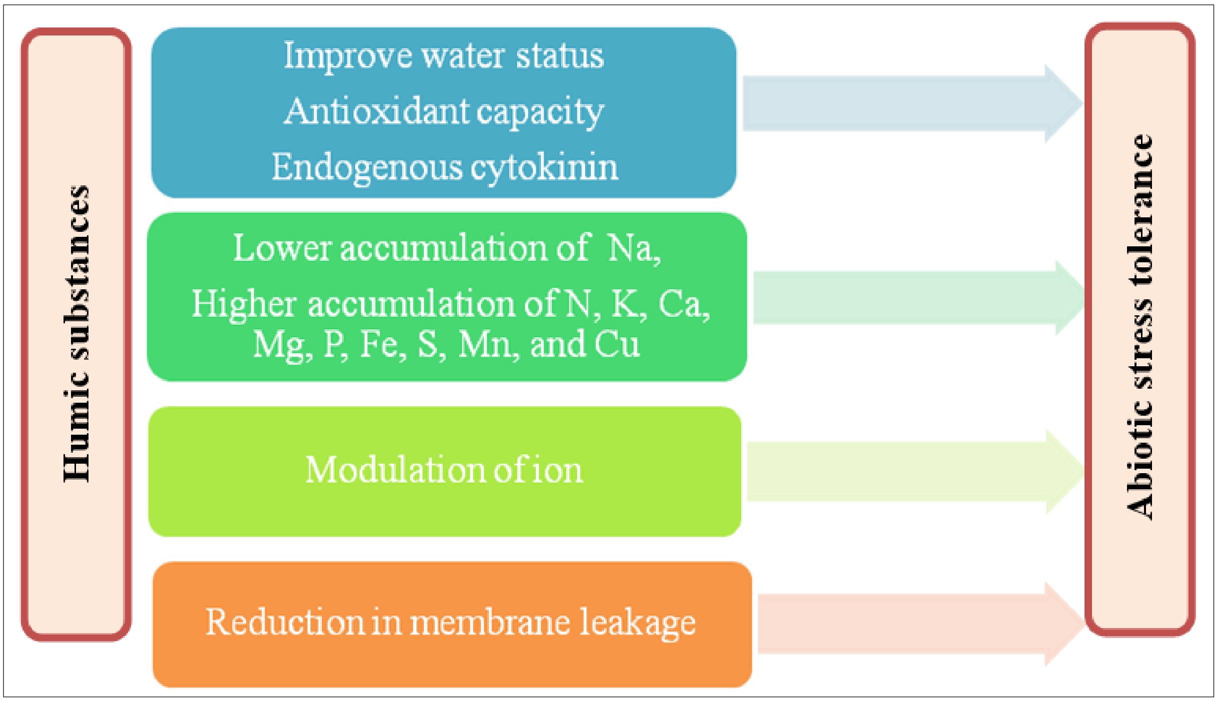

Fig 1: Mechanisms for improving plants' resistance to abiotic stress by Humic substances (Nagachandrabose& Baidoo, 2021) [9, 10]

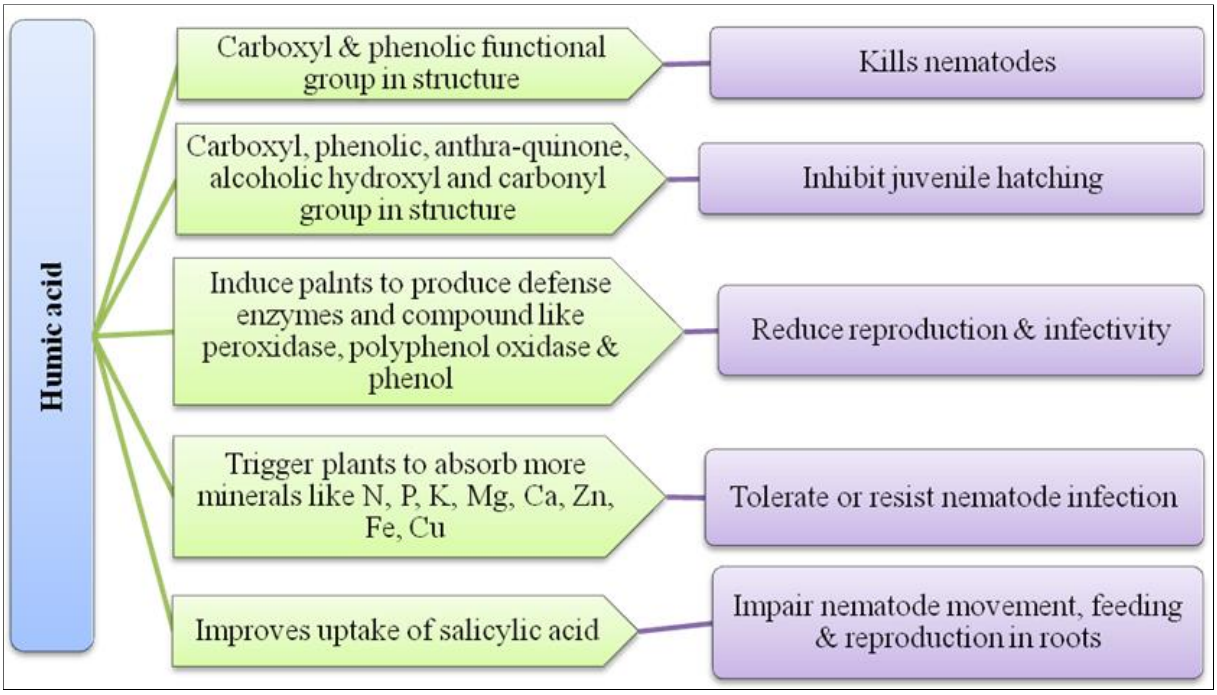

Fig 2: Mechanisms of humic acid in the nematode control

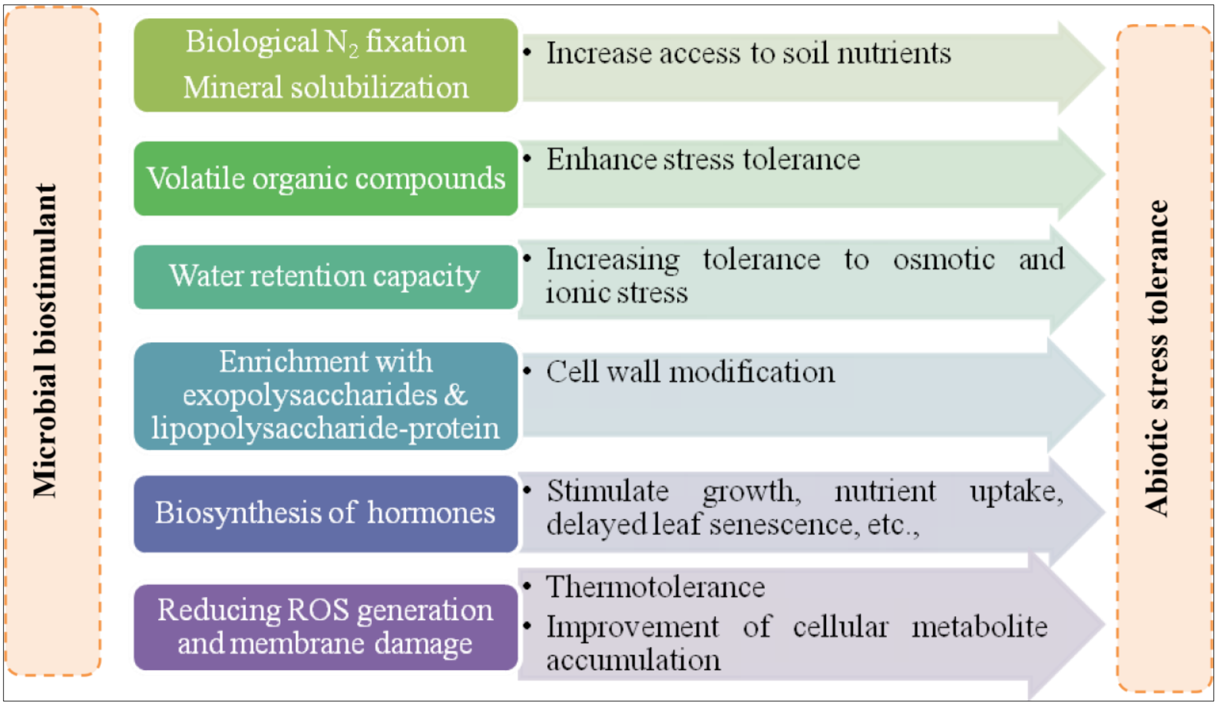

Fig 3: Mechanisms for improving plants' resistance to abiotic stress by Microbial biostimulant (Hasanuzzaman, *et al*., 2021) [17]

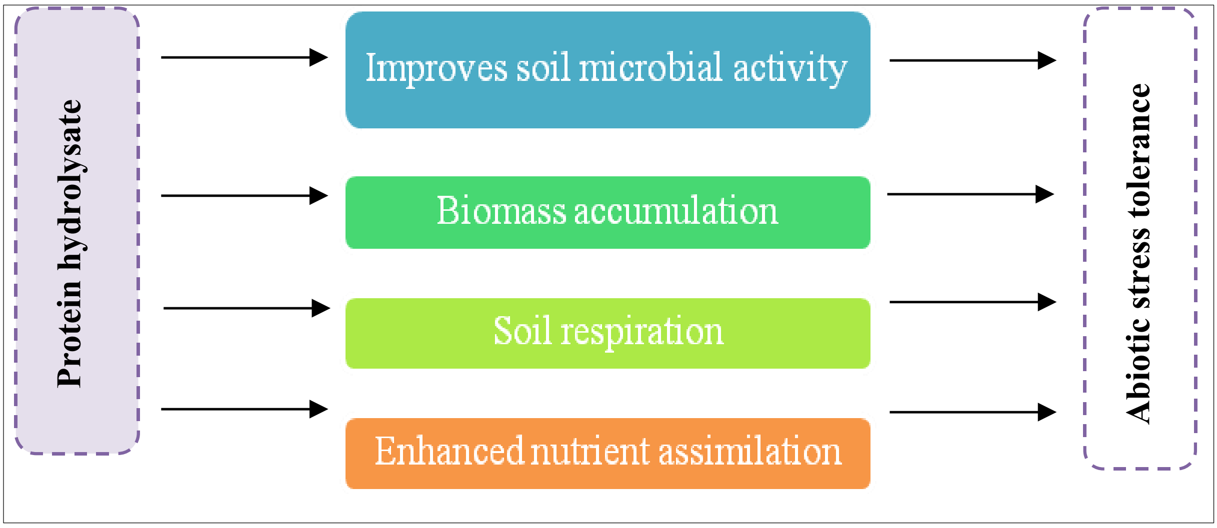

Fig 4: Mechanisms for improving plants' resistance to abiotic stressby Protein hydrolysate (Hasanuzzaman, *et al*., 2021) [17]

Role of biostimulant in abiotic stress management

Salinity stress mitigation

Humic acid (300 ppm) immersed nutrient media serves as best media to boost the growth and yield of strawberry even under salinity. (Saidimoradi, *et al*., 2019) [11]. Under salt stress conditions, accumulation of Na+ is higher than the K+ in shoot and root which shows that more Na+ has been observed by root and translocated from root to shoot by means of transpiration stream. Application of Humic acid leads to absorption of sodium by humic complex and subsequently leached through water, increasing the potassium availability to the root. Futher more, Salt stress increased the amount of leaf necrotic tissue, the activity of antioxidant enzymes, hydrogen peroxide, and lipid peroxidation; however, these features were recovered and the salt tolerance index was raised by adding humic acid to nutritional solutions. A typical increase in proline content can be considered as one of the tolerance capacity criterion. But Saidimoradi, *et al*., (2019) [11] observed that plants treated with humic acid had lower levels of proline, which may be related to a mechanism for reducing the negative effects of salinity.

Water Stress management

Protein hydrolysates, a substance made from protein sources through partial hydrolysis and are composed of mixtures of polypeptides, oligopeptides, and amino acids, that have been proven to mitigate the effects of stress by promoting the growth of younger vegetative organs and reducing the degree of cell dehydration when applied to roots before imposing water restriction. Furthermore, the plants treated with the biostimulant displayed a larger accumulation of biomass and higher berry diameter both in ideal water circumstances and during water stress. (Meggio, *et al*., 2020) [7].

Mitigation of Heat Stress

The use of microorganisms that lower ethylene emission has a lot of potential since lowering ethylene under stress conditions could prevent the detrimental effects of heat stress on plant growth. Bacillus cereus, which produces ACC-deaminase, assisted in reducing ethylene production by cleaving the ACC to -ketobutyrate and ammonia and reducing the negative effects of ethylene on plant growth under heat stress. Additionally, in both normal and stressful circumstances, Bacillus cereus demonstrated the ability to produce Exopolysaccharides (EPS). These EPS-producing bacteria have the capacity to give plants protection from abiotic stress. (Mukhtar, *et al*., 2020) [8].

Role of biostimulant in biotic stress management

Pest management

Nagachandrabose, *et al*., (2021) [9, 10] found that NSKE has potential to reduced T. semipenetrans population by 56.8–58.1% in soil and 46.4–56.2% in root. More anti-nematode chemical components, such as alkaloids, saponins, triterpenoids, steroids, tannins, phenol, anthocyanins, flavonoids, and glycosides, are present in NSKE. Additionally, it was discovered that the purified azadirachtin component produced from NSKE has nematicidal, nematostatic, and egg inhibitory effects against nematodes. Furthermore, NSKE has the ability to release nitrogen gradually by inhibiting the soil nitrification process through the metabolite of its triterpenes.

Disease management

The application of fulvic acid significantly lowered the severity of downy blight in table grapes by inducing phenylpropanoid metabolism, as shown by the accumulation of phenolic and flavonoid compounds, higher activity of phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), and 4-coumarate-CoA ligase (4CL), and up-regulation of genes involved in phenylprop (PAL, C4H, 4CL, STS, ROMT and CHS). Xu, *et al*., (2019) [14] found that the fulvic acid treatment significantly raised the content of total phenolics and flavonoids during storage compared to the control, and the level of total phenolics was 38.18 percent greater in berries treated with fulvic acid than controls at 36 hours after pathogen inoculation, which were proven to be disease resistant.

Role of biostimulant in nutrient uptake efficiency

The mechanisms through which biostimulants influence nutrient uptake are typically connected to an improvement in the soil physico-chemical properties, solubility of nutrient, root morphology, and arbuscular mycorrhizal fungi colonisation of the roots. Mattner *et al*., (2018) [6] observed that strawberry plants treated with seaweed extracts (Duvillaeapotatorum and A. nodosum) had longer and denser roots, hinting that the crop was using nutrients more effectively. Seaweeds can be utilised as aorganic source of fertilizer. They improve mineral composition of plant tissues especially micro nutrient *viz*., Fe, Zn, Mg, Cu. Soppelsa, *et al*., (2019) [12]. Similarly, the yield gap is further decreased by microbial biostimulant-induced plant growth enhancement due to improved biological N2 fixation, solubilization of minerals and other nutrients, and increased plant access to soil nutrient resources even under unfavourable environmental conditions.

Role of biostimulant in growth, productivity and quality

Bio stimulants plays a major role in increasing plant growth, production and productivity of horticultural crops. The foliar spray of humic acid (60 ml/L) at bud burst stage and soil drenching of bio-inoculants (PSB and A. chroococcum @ 10 ml/plant) accompanied with 80% recommended dose of fertilizer uplift the growth and traits which enhance flowering, fruiting, and other yield and quality related traits. (Ashwini, *et al*., 2022) [2]. Likewise, humic and fulvic acid spray of 0.15% at just before the pink bud stage and the pea size stage of apple increase the yield and lead to highest production of organic apple. (Khan, *et al*., 2019) [18]

Role of biostimulant in plant physiological functions

Biostimulants tend to have greater effects on physiological functions of plants. The combination of humic substances and rhizobacteria applied at every 15 days and 30 days respectively improved fruit physiology and increased the yield of crop. The application of fulvic Acids (3 mL) + Azospirillum brasilense (5 mL) increased the number of leaves (38.3%), root volume (42.6%), the fresh weight (130%) and dry weight (63.8%), the number of fruits (50.0%) and the yield (59.5%) in Strawberry plants cultivar 'San Andreas'. (Cruz, *et al*., 2022). In addition the fulvic Acids (3 mL) and Pseudomonas fluorescens (5 mL) combination able to favor photosynthesis (127.3%) and to increased TSS (25%). Similarly the mixture of humic and fulvic acids (3 mL) + Azospirillum brasilense (5 mL) increased the content of Phenols in 20%. Thus, the humic compounds and rhizobacteria are an environmentally friendly alternative to be employed as a biostimulant in the growth and quality of plants.

Role of biostimulant in management of physiological disorder

Seaweed extract has the potential to showed a significant enhancement infruit weight, yield, retention of fruits on tree, and fruit quality. It is evident from the research finding of Dash, *et al*., (2021) [4]. The reduction in fruit drop and improvement over the quantity and quality of mango can be achieved through three foliar spray of Ascophyllum nodosum extract (3000-5000 ppm) at the panicle initiation, pea, and marble stages of fruit growth. Variations in temperature or humidity during flowering, pollination, fruit set, or maturation, exhaustion of tree reserves during the heavy crop load period, vigorous vegetative growth with high gibberellin levels during flower bud differentiation period, and carbon: nitrogen (C/N) ratio imbalance have all been identified as primary factors that contribute to alternate bearing in mango. The morphological, physiological, biochemical, and genetic processes in plants can be effected by humic and boric acid both in direct and indirect ways. These effects eventually have an impact on plant development, yield, and fruit quality. The combinational spray of 0.30 percent humic acid and 600 mg/L boric acid on the "Zebda" mango had a significant impact on uptake of nutrient uptake, assimilation of carbon, phytohormone activity and also on carbon metabolism, suggesting that the tree may be able to withstand unfavourable conditions that result in floral malformation and lower productivity. (El-Hoseiny, *et al*., 2020) [19].

The foliar applications of humic acid and boric acid combinations bring the hormonal balance in plants by the increased level of auxin, gibberellin and cytokinin with decreased level of ABA and there by reduced the mango malformation. In addition, humic acid and boric acid application has a negligible effect on the occurrence of alternate bearing by the increase in carbohydrates and osmostimulants which may be directly resulted the higher rate of photosynthesis. Increased carbohydrate availability is needed for a high C/N ratio, which is essential for floral initiation in mango. (Upreti *et al*., 2013) [13]. Increased carbohydrate availability is required for a high C/N ratio, which is a key factor in mango floral initiation. This ratio can also influenced by the environmental conditions and prevailing metabolic balance.

Future thrust areas

Many research works are required for confronting a number of open questions related to biostimulants, such as: at what extend applied management practices affect these effects? How much days the plant biostimulant have persistence effects? How can the different components of biostimulants be accounted for in developing novel and specific biostimulant products? Finding answers to these questions creates wider scope for research in this field. Furthermore, a well-defined mechanism of action of biostimulants in plants are not studied in detail and thus insights on molecular mechanisms of action are needed.

Conclusion

Any substance or microorganism applied to crops or soils with the intention of increasing plant nutrient efficiency, tolerance to abiotic stress, and/or crop quality traits despite its nutrient status is referred to as a plant biostimulant which proves that it has wise potential to serve as efficient tool in sustainable horticulture. Even though biostimulants known to mankind for years the insights on the mechanism they work are lagging till now and this will create a wide scope for research on biostimulants in future.

References

1. Afonso S, Oliveira I, Meyer AS, Gonçalves B. Biostimulants to Improved Tree Physiology and Fruit Quality: A Review with Special Focus on Sweet Cherry. Agronomy. 2022 Mar 9;12(3):659. https://doi.org/10.3390/agronomy12030659
2. Ashwini N, Kumar P, Joshi AK, Sharma NC, Sharma N, Sharma N. Synergistic action of humic acid substances and bio-inoculants in guava (*Psidium guajava* L.): impact on growth traits, fruiting, nutrient profiling and rhizosphere stochiometry in meadow rainy season plant-soil interface. Journal of Plant Nutrition; c2022 Feb 23. p. 1-5. https://doi.org/10.1080/01904167.2022.2046069
3. Billard V, Etienne P, Jannin L, Garnica M, Cruz F, Garcia-Mina JM, *et al*. Two biostimulants derived from algae or humic acid induce similar responses in the mineral content and gene expression of winter oilseed rape (*Brassica napus* L.). Journal of plant growth regulation. 2014 Jun;33(2):305-16.
4. Dash A, Samant D, Dash DK, Dash SN, Mishra KN. Influence of Ascophyllum nodosum extract, homobrassinolide and triacontanol on fruit retention, yield and quality of mango. Journal of Environmental Biology. 2021 Jul 1;42(4):1085-91.
5. Dobbelaere S, Croonenborghs A, Thys A, Vande Broek A, Vanderleyden J Phytostimulatory effect of Azospirillum brasilense wild type and mutant strains altered in IAA production on wheat. Plant and soil. 1999 May;212(2):153-62.
6. Mattner SW, Milinkovic M, Arioli T. Increased growth response of strawberry roots to a commercial extract from *Durvillaea potatorum* and *Ascophyllum nodosum*. Journal of applied phycology. 2018 Oct;30(5):2943-51.
7. Meggio F, Trevisan S, Manoli A, Ruperti B, Quaggiotti S. Systematic Investigation of the effects of a novel protein hydrolysate on the growth, physiological parameters, fruit development and yield of grapevine (*Vitis Vinifera* L., cv Sauvignon Blanc) under water stress conditions. Agronomy. 2020 Nov 14;10(11):1785.
8. Mukhtar T, Rehman SU, Smith D, Sultan T, Seleiman MF, Alsadon AA, *et al*. Mitigation of heat stress in *Solanum lycopersicum* L. by ACCdeaminase and exopolysaccharide producing Bacillus cereus: effects on biochemical profiling. Sustainability. 2020 Mar 11;12(6):2159.
9. Nagachandrabose S, Baidoo R. Humic acid–a potential bioresource for nematode control. Nematology. 2021 Jul 7;24(1):1-0.
10. Nagachandrabose S, Jayaraman J, Somasundaram P. Effect of application of organic bio-products through drip irrigation against *Tylenchulus semipenetrans* in citrus. Indian Phytopathology. 2021 Dec;74(4):993-1000. https://doi.org/10.1007/s42360-021-00379-3
11. Saidimoradi D, Ghaderi N, Javadi T. Salinity stress mitigation by humic acid application in strawberry (*Fragaria x ananassa* Duch.). Scientia Horticulturae. 2019 Oct 15;256:108594. https://doi.org/10.1016/j.scienta.2019.108594
12. Soppelsa S, Kelderer M, Casera C, Bassi M, Robatscher P, Matteazzi A, Andreotti C. Foliar applications of biostimulants promote growth, yield and fruit quality of strawberry plants grown under nutrient limitation. Agronomy. 2019 Aug 26;9(9):483.
13. Upreti KK, Reddy YT, Prasad SS, Bindu GV, Jayaram HL, Rajan S. Hormonal changes in response to paclobutrazol induced early flowering in mango cv. Totapuri. Scientia Horticulturae. 2013 Feb 4;150:414-8.
14. Xu D, Deng Y, Xi P, Yu G, Wang Q, Zeng Q, *et al*. Fulvic acid-induced disease resistance to Botrytis cinerea in table grapes may be mediated by regulating phenylpropanoid metabolism. Food chemistry. 2019 Jul 15;286:226-33.
15. Jindo K, Olivares FL, Malcher DJ, Sánchez-Monedero MA, Kempenaar C, Canellas LP. From lab to field: Role of humic substances under open-field and greenhouse conditions as biostimulant and biocontrol agent. Frontiers in plant science. 2020 May 12;11:426.
16. Liu J, Huang J, Fan KW, Jiang Y, Zhong Y, Sun Z, Chen F. Production potential of Chlorella zofingienesis as a feedstock for biodiesel. Bioresource technology. 2010 Nov 1;101(22):8658-63.
17. Sachdev S, Ansari SA, Ansari MI, Fujita M, Hasanuzzaman M. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. Antioxidants. 2021 Feb 11;10(2):277.
18. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arabian journal of chemistry. 2019 Nov 1;12(7):908-31.
19. Elsheery NI, Helaly MN, El-Hoseiny HM, Alam-Eldein SM. Zinc oxide and silicone nanoparticles to improve the resistance mechanism and annual productivity of salt-stressed mango trees. Agronomy. 2020 Apr;10(4):558.