A Seminar Paper

On

"Biostimulants Efficacy on Abiotic Stress Mitigation of Horticultural Crops"

Course Title: Seminar Course

Code: AFE 598

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Biostimulants Efficacy on Abiotic Stress Mitigation of Horticultural Crops[1]

By

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ABSTRACT

Plant biostimulants, or agricultural biostimulants, include diverse substances and microorganisms that enhance plant growth, abiotic stress tolerance and crop quality traits, regardless of its nutrient content. Horticultural crops are currently exposed to multiple abiotic stresses such as drought, heat, salinity, and nutrient deficiencies due to change of global environment explosively. This can strongly reduce crop performance, causing increasing losses in terms of yield. Biostimulants including bioactive molecules that improve the general health, vitality, and growth of plants; protect them against these stresses and are successfully used in horticultural crops. This review investigates the concept and significance performance of biostimulants in mitigating different abiotic stresses, their mechanisms and the impact of application for improving nutrient uptake and utilization efficiency of horticultural crops. The effect raise by biostimulants are frequently delicate to identify and are still under exploration. They can act on plant productivity as a direct response of plants or soils to the biostimulant application (foliar/spray) or an indirect response on the soil and plant microbiome with subsequent effects on plant productivity. Biostimulants offer a potentially novel approach for the regulation and modification of physiological processes to stimulate growth, to mitigate stress induced limitations, and to increase yield of horticultural crops. In this manuscript, the available information will be reviewed regarding the implicit use of biostimulants on horticultural crops under environmental stress conditions to alleviate abiotic stress and increasingly being introduced in conventional crop production to respond to profitable and sustainability imperatives.

Keywords: Biostimulants, abiotic stress, horticultural crops, mitigate, crop growth, production

1 Title of the seminar paper presented as a part of course, HRT 598 during Winter’2022

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Biostimulants are the compounds that produce non-nutritional plant growth responses and reduce stress by enhancing stress tolerance. This is in contrast to fertilizers, which produce a nutritional response. A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutritional efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content (Du Jardin *et al*., 2015). They improve plant function, promote plant growth and productivity, interact with several processes involved in plant responses to stress, and increase the accumulation of antioxidant compounds that allow decrease in horticultural crops stress sensitivity (Van Oosten *et al*., 2017).

Plants are continuously expressed to adverse environmental conditions. The modification of the raising environment caused by climate change is constantly adding the pressure upon horticultural crops cultivation with seasonal climatic conditions characterized by a former incidence of extreme abiotic events (Andreotti, 2020). It is become more necessary to develop horticultural crops in order to supply the world's rapidly expanding population with the fruits, vegetables, and other things they need. Tolerance to abiotic stressors is a crucial characteristic of horticultural species because of their marketing value is generally advanced than field crops, having more resources for farming and they also give a source of numerous nutrients, fibre, minerals, and carbohydrates, which are essential in a healthy diet (Shannon *et al.*,1998). About 90% of essential vitamin C and 60% of vitamin A for human comes from vegetables, which is reported by Food and Agriculture Organization (FAO). In effect, low fruit and vegetable intake is a main contributing threat measure to various wide and enfeeble nutritive diseases.

Abiotic stresses like high temperature, drought, salinity are key challenges for horticultural crops and also responsible for severe reduction of both yield and quality of fruit and vegetable production (Mall *et al*., 2021). (FAO) reported that only 3.5% of the global land area is not affected by some environmental constraints. The American Society for Horticultural Sciences' working groups on stress physiology and vegetable breeding put a special emphasis on improving horticultural crops' ability to withstand abiotic stress while taking the effects of climatic change into account in 2010 at the society's periodic conference (Mou, 2011). Bisbis *et al*., (2018) investigated the double effects of elevated temperature and increased CO on the physiology of different vegetables. They looked at potential adaption techniques while observing various reactions based on the types of crops and the speed of the stress that can be enforced in order to mitigate the pressure of environmental change. Even now, most studies on climate change impacts concentrate on major crops, and only few papers pay attention to fruits and vegetables in terms of production, quality, and supply chain (Parajuli *et al.*, 2019; Bisbis *et al.*, 2018).

The horticulture industry is consequently actively looking for innovative agronomic methods that may be used to mitigate the harmful effects of environmental pressures while maintaining the overall sustainability and quality of the output as it is a delicate task of preventing damage caused by abiotic stresses. Among different new bio-technological inventions, biostimulants have acquired increased sight in the last decade also because of their ability to exploit agricultural, urban, and industrial waste materials in a perspective of circular economy and environmentally friendly (Du Jardin *et al.*, 2020).

Biostimulants are generally applied to high-value plants, substantially greenhouse plants, fruit trees, outdoor vegetables, flowers, and other ornamentals of horticultural plants, to increase yield and product quality in a sustainable manner. Numerous horticultural companies are investing in the development of new biostimulant products and the development of the most effective bioactive molecules capable of eliciting specific plant responses to abiotic stresses. According to several investigations, when given to horticultural crops, such as vegetable and fruit crops, when they truly need to be protected, biostimulants or specialized bioactive chemicals can also induce plants to tolerate stress (Van Oosten *et al.*, 2017; Yamauchi, 2018).

Effects are being evaluated in relation to horticultural crop cultivation uses of biostimulants to mitigate abiotic stresses from different points of view in order to demonstrate that the use of these substances can be an effective and sustainable tool in horticultural production.

By considering the above situation, this review paper is meet with the following objectives:

- To evaluate the performance of biostimulants in mitigating abiotic stress in horticultural crops, including salinity, water stress, cold, high temperature, and other stress factors.

- To illustrate the mechanisms of biostimulant action and impact on the yield and quality of horticultural crops under different abiotic stress conditions.

CHAPTER II

MATERIALS AND METHOD

The main focus of this seminar paper is review. So, there is no specific method involved to collect the data. All of the information has been collected from the secondary sources such as various relevant journals, paper and articles that have been already published. The data was also collected from different relevant reports, and websites which are available on online platforms.

I was able to make this paper better with the help of valuable criticism and suggestions from my major professor, and the course instructors. Afterward, all of the collected information was arranged according to the sequence and presented in this paper for better understanding and clarification.

CHAPTER III

REVIEW OF FINDINGS

3.1 Biostimulants and Crop Sresses

The term biostimulant is inadequately includes numerous products that have similarly been described as biogenic stimulants, metabolic enhancers, plant strengtheners, positive plant growth regulators, elicitors, allelopathic preparation, plant conditioners or phytostimulators. They may be organic or inorganic substances that contain bioactive elements and/or microorganisms, which, when applied to a plant or rhizosphere, promote plant growth and productivity by increasing nutrient uptake and assimilation rates, stress tolerance, and/or product quality, regardless of the amount of nutrients present. (Bulgari *et al*., 2019; Rouphael *et al*., 2020).

Plants are repeatedly subjected to a confluence of stressful events, from seed germination to the whole life stage. Stress caused by unfavorable stimuli can significantly affect crops and reduce yields due to plants respond by using their energy reserves to fight stress instead of concentrating on yielding. Depending on the type of the trigger factor, these stresses are frequently split into two categories- biotic and abiotic stresses. (Franzoni *et al*., 2022). Biotic stress caused by living organisms, including insects, bacteria, fungi, and weeds that affect crop development and productivity. Abiotic stress that are generally associated with the climatic, edaphic, and physiographic part of the environment, when they are bounding factors of crop growth and survival (Franzoni *et al*., 2022). The most serious abiotic stresses in agricultural productivity, around all over the world, are drought, salinity, non-optimal temperatures, and low soil fertility (Verma, 2016).

Biostimulators are recommended as an intervention method to be applied in case of stress environment, e.g., black frost, drought, hail, strong wind, and chemical contamination with herbicides/pesticides. Due to their diversity and/or complexity, precise mechanisms of biostimulator effects are difficult to identify, but certain significant paths have been founded also (Source: Posmyk *et al*., 2016).

3.1.1 Biostimulants into Categories and Their Effect on Abiotic Stresses of Horticultural Crops

Over the times, number of authors have mentioned various groups of biostimulants based on their nature, key components or mode of activities. Du Jardin, (2015) just completed a thorough analysis of the international scientific literature on plant biostimulants as part of a deal with the european commission. The author mentioned 8 groups of substances that conduct as biostimulants: humic substances, complex organic materials, beneficial chemical elements, inorganic salts including phosphite, seaweed extracts, chitin and chitosan derivates, antitraspirants, and amino acids in free radical and Nitrogen containing substance.

Based on the nature of the raw ingredients, biostimulants can currently be divided into the following primary groups. - Humic Substances, Seaweeds and Plant Extracts, Hydrolyzed Proteins and Nitrogen-Containing Compounds, Microorganisms, Glycine betaine -Inorganic Compounds and also chitosan.

Abiotic stress is defined as environmental circumstances that minimize the growth and yield below optimum equilibrium such as cold, drought, and salinity which has been becoming a major threat to horticultural food security and crop productivity. Crops can start a variety of molecular, cellular, and physiological changes to react to and adapt to abiotic stress (Huang *et al*., 2013). Various number of studies have been introduced to assess the usefulness of applying biostimulants to raise plant development when challenged to abiotic conditions.

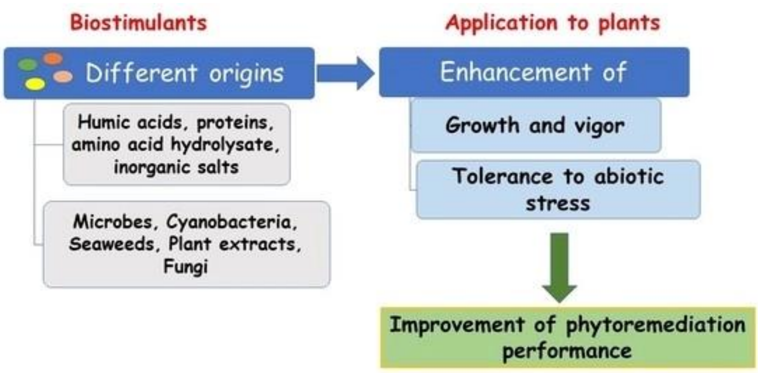

Figure 1. Use of biostimulants (Source: Bartucca *et al*., 2022).

Abiotic stress causes an inconsistency of pro-oxidant and also antioxidant composites in horticultural crops, also known as oxidative stress, it usually happens when a stressful event upsets the equilibrium between the product of ROS-reactive oxygen species, the quenching exertion. Environmental stress factors and the presence of heavy metals such cadmium, copper, and chromium, elicit stress responses in plants, and reduced photosynthetic activity, degradation of chlorophyll, destruction of an organic molecule, and damage to the lipid peroxidation which ultimately lower plant growth and thus crop yields. Their volume dramatically increases under abiotic stress and, if not controlled could hamper in cellular damage and causes plant death. Abiotic stress poses a threat to the development and productivity of horticulture crops today and is to blame for significant crop losses on a global scale. Biostimulants are now used more into production systems in an effort to minimize productivity losses by altering physiological processes in plants (Yakhin *et al*., 2017). Biostimulants are often created by combining natural or synthetic chemicals made of various hormones or plant hormone precursors that acts directly on the physiological mechanism to provide potential favor for growth, development, and/or make reaction on water stress, saline, and toxic elements, such as toxic aluminum (Du Jardin, 2015).

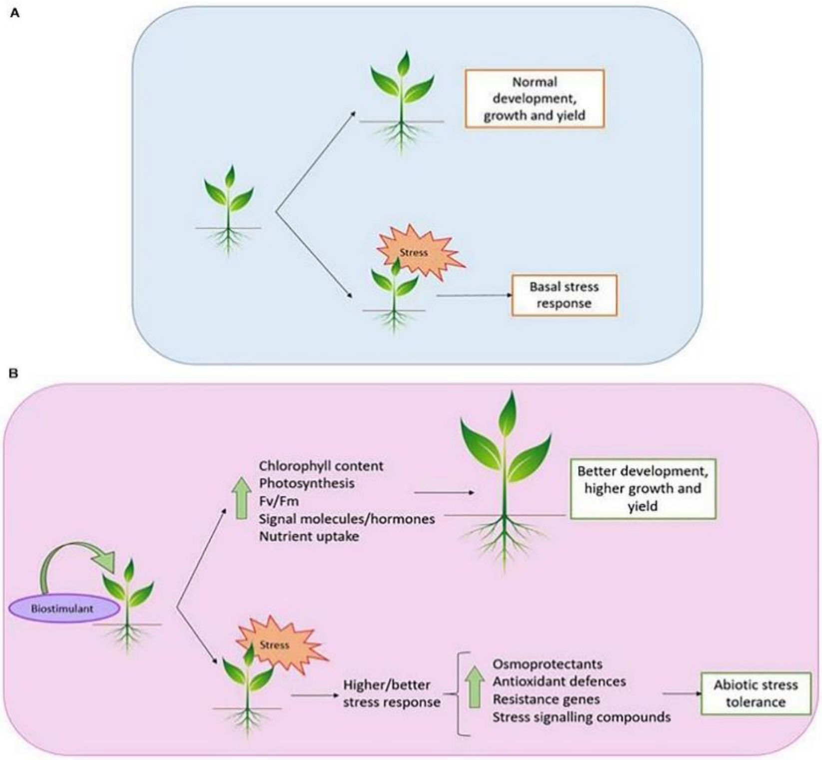

Figure 2. Plant response against abiotic stress. Without **(A)** and with biostimulant **(B)**. (Source: Garcia-Garcia *et al*., 2020).

When biostimulants are used to a plant, seed, soil or growing media - in confluence with established fertilization scheme, promote the crop's nutrient use effectiveness, or give rise other visible or circular benefits to plant development or stress response (Beaudreau, 2013).

3.2 Effect of Biostimulants on Horticultural Crops

In recent years, a variety of new sorts of techniques have been developed to enhance sustainable production in horticulture crops, use of biostimulants is one of them.

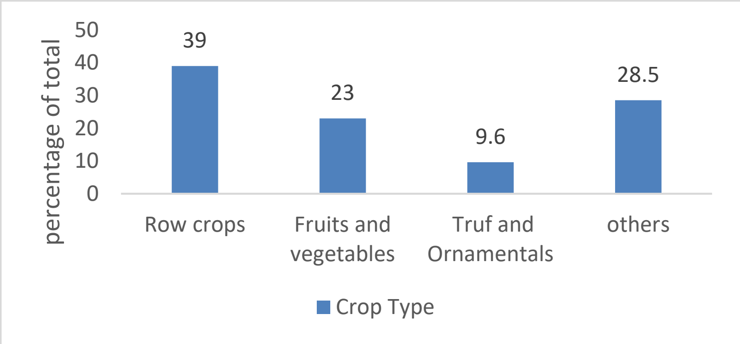

Figure 3. Biostimulant application in different crops (Source: Critchley *et al*., 2021).

There is a positive effect of biostimulants on the growth of fruits, vegetables, ornamentals or other horticultural crops based on humic, fulvic, and carboxylic acids, Hydrolyzed proteins and amino acids containing products, microorganisms and other elements on the yielding has been proven.

Biostimulants raise flowering, vegetative growth and fruit production per plant, but there is few information on them. Some of the most prominent findings on the effect of different biostimulator in improving fruit crop especially focused on growth, yield, and fruit quality (Rana *et al*., 2022). It is crucial to increase natural resource productivity through the application of bio-stimulants in vegetables in order to improve sustainable food production, food and nutritional security, and environmental degradation caused by pesticide use.

Table 1. Indicative list of some horticultural crops with positive effect of biostimulants are currently applied**.**

Humic substances (Humic acid & Fulvic Acid)	Seaweed extracts	Protein hydrolysates	Microorganisms
Apricot	Mango	Grapevine	Strawberry
Strawberry	Banana	Banana	Tomato
Tomato and cucumber	Cauliflower	Tomato	Citrus
Eggplant	Carrot		Portulaca
Broccoli	Cabbage		Rose

Mango	Lettuce

(Source: Drobek *et al*., 2019 Canellas *et al*., 2015)

To enhance the sustainability of vegetable production, bio-stimulants can be effectively used. The use of plant biostimulants is becoming more popular, driven by the growing interest of growers in natural materials and beneficial microorganisms that can sustainably increase the productivity of vegetables and ornamentals. Some ornamental horticulture plant-Bougainvellea, rose, salvia, portulaca, strawflower, zinnia etc have also effective use of biostimulants.

The use of biostimulants in horticulture-vegetables, fruits, vines including ornamentals, more resilient to climate change and create more livable, environmentally friendly, and sustainable agricultural production.The study presented here confirms the advantages of biostimulants application in horticultural production, especially in stressful growth conditions due to transplantation, reduced fertilization, or other abiotic stress incidences.

3.3 Performance of Biostimulants on Different Abiotic Stresses

3.3.1 Cold or Chilling Stress

The lowering of temperature or the return of cold temperatures in late spring is one of the most dangerous abiotic stresses seriously affecting production (Ferrante *et al*., 2018). Low temperatures reduce plant metabolism and delay physiological responses. A reduced metabolism, consequent to cold stress, leads to an inhibition of the exertion of photosystem II, called photoinhibition. The performance of biostimulants against cold stress usually increases the accumulation of osmotic molecules, that also increase membrane thermostability, decrement the chilling injury.

In Strawberry during cold stress condition use of biostimulants have significant effect on their growth and development.

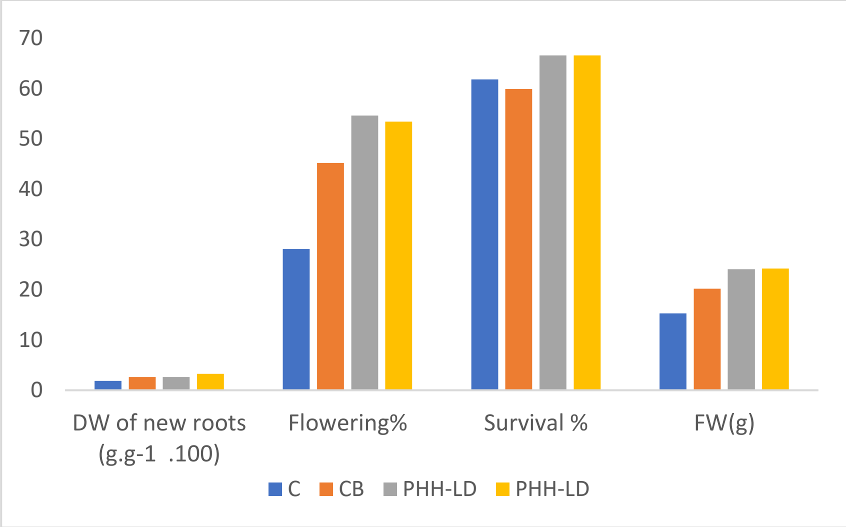

Figure 4. Biostimulants effect on strawberry during cold stress (Source: Marfa *et al*., 2008).

Means and statistical separation corresponding to the following parameters: ratio of the dry weight of new roots to the total dry weight of roots; percentage of plants in flower; percentage of live plants; and accumulated fresh fruit weight of strawberry plants (FW) subject to the different treatments: C- control, CB-commercial biostimulant, PHH-LD-porcine hemoglobin hydrolysate and PHH-HD porcine hemoglobin hydrolysate.

In tomato, cold tolerance has been promoted by the use of psychrotolerant soil bacteria which showed higher seeds germination, reduced membrane damage, and antioxidant systems activation (Subramanian *et al*., 2016). These soil bacteria can be considered as apparent biostimulants for protecting plants against cold stress. Pepper (*Capsicum annuum*) seedlings were given a treatment with 5-aminolevulinic acid in order to promote chilling tolerance through different ways (soaking, spraying or drenching). BactorS13 or Flortis Micorrize has demonstrated the capacity to increase osmolytes in order to overcome the decline in plant development caused by cold stress. (Miceli *et al*., 2021). Excellent results are found from biostimulants based on algae, amino acids, or microbials that can induce protection against low temperatures, by the accumulation of cryoprotective substances and/or the activation of membrane repair systems (De Saeger *et al*., 2020).

3.3.2 Drought Stress

Water stress is one of the most common abiotic stresses occurring in horticultural crops in different cultivation areas. Drought stress explosively influences plant gas exchange changing photosynthetic and transpiration rates, which are directly linked to yield. There are several strains of bacteria colonizing soil promoting plant growth through its metabolic activities and plant interactions. They produce exopolysaccharides, phytohormones, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, volatile compounds, converting several metabolic plant responses as accumulation of osmolytes and antioxidants, or up or down regulation of stress responsive genes and alteration in root morphology leading to a tolerance of water stress (Kumar *et al*., 2018).

Table 2. Application of biostimulants in water stress, affected Fv/Fm values significantly and had a considerable influence on yield, Electron transport rate (ETR), and Chlorophyl content index (CCI) of broccoli.

Treatment	Initial fluorescence (Fo)	Maximum fluorescence, (Fm)	Maximum photochemical efficiency (Fv/Fm)	Quantam yield
Control	2.75	1.20	4.45	33.66
Biostimulant	4.17	3.40	12.58	7.46

Source: Kahuzewicz *et al*., 2018)

The crop's water balance can be improved by biostimulants, which can also boost root water absorption capacity and, ultimately, reduce losses. (Del Buono *et al*., 2021). Leaf yellowing is another common symptom of drought stress due to chlorophyll degradation. An excellent product for the development of leafy vegetables, *A. nodosum* biostimulants treatments enhance the green color under stress conditions by enhancing the biosynthesis and lowering the breakdown of chlorophyll. After applying biostimulants, a decrease in water loss, wilting damage, and 3-carbon dialdehyde MDA was seen.

3.3.3 Salinity Stress

Among abiotic stresses, salinity is one of the primary damaging factors affecting plant growth and metabolism as an effect of osmotic stress caused by salt. Sodium chloride (NaCl) is the more abundant salt presents in saline environments and is toxic in higher concentrations (Bulgari *et al*., 2019). Bioactive compounds present in seaweed extracts are able to improve plant tolerance against abiotic stresses too. The application of biostimulants in situations of salinity stress results in the accumulation of osmolytes, which improves the osmotic potential of the cell and the positioning of molecules that protect it from oxidative stress.

There is a positive effect of in spinach, when propolis extract (PE) used as seed presoaking agent to increases the plant height, number of leaves per plant, total leaf area, and fresh weight of leaves per plant in salty conditions.

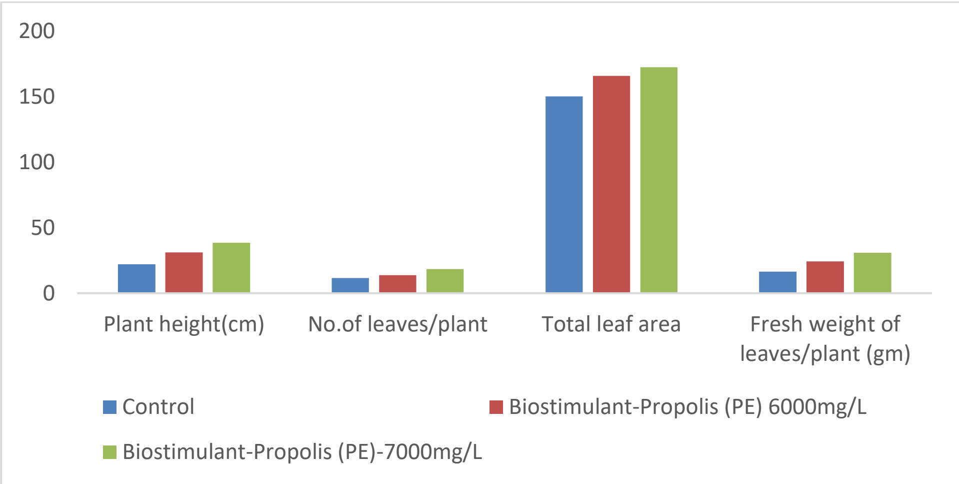

Figure 5. Effect of propolis extract (PE) on growth characters-spinach on saline soil (Source: El-Yazal *et al*., 2019).

Table 3. Growth characteristics of use of Biostimulant (*G. mosseae*) and non-AMF trifoliate Citrus-orange (*Poncirus trifoliata*) seedlings under NaCl stress

Salt stress	**Biostimulant-Microorganism**	**Plant height(cm)**	**Stem diameter(cm)**	**Leaf number**	**Leaf area**
0	AMF-*G.mosseae*	23	0.246	21.8	19.4
	Non-AMF	15.6	0.206	15.8	15.7
100	AMF-*G.mosseae*	22.1	0.225	19.9	18.0
	Non-AMF	13.6	0.191	12.5	12.4

*AMF=Arbuscular mycorrhizal fungi (Source: Wu *et al*., 2010)

The table indicates significant difference among treatment of AMF and Non-AMF biostimulant.

The content of proline, simple sugars, alcohols, abscisic acid, and antioxidant substances that can prevent damage may rise as a result of the use of biostimulants (Carillo *et al*., 2020).

Like this, a recent study highlighted the capability of a freak-honey based biostimulant to ameliorate the tolerance of onion plants to salinity stress; showed higher biomass, bulb yield, and photosynthetic colors. Several trials have been carried out using different PGPR that are suitable to enhance abiotic stress tolerance. Inoculation with *Azospirillum brasilense* showed positive results on lettuce (Fasciglione *et al*., 2015), sweet pepper (Del Amor *et al*., 2011) grown under salty condition.

3.3.4 High Temperature/ Heat Stress

High temperatures can also cause damage to horticultural crops as a result of differences of cell membranes and enzymatic functionalities. Most serious damage can be obtained in areas with temperatures above 37 ◦C (Ferrante *et al.,* 2018). The range between 30 ◦C and 45 ◦C is the optimal temperature for structural integrity and enzymatic activity, which are irreversibly denatured when temperature increases above 60 ◦C.

Tomato is considered one of the most sensitive species to non-optimal temperatures, after treatments, show good result - membrane damage, measured as electrolyte leakage, MDA and H2O2 levels was decreased in this vegetable crops. The following graph show positive effect on tomato fruits composition treated with the biostimulant CycoFlow applied by fertigation.

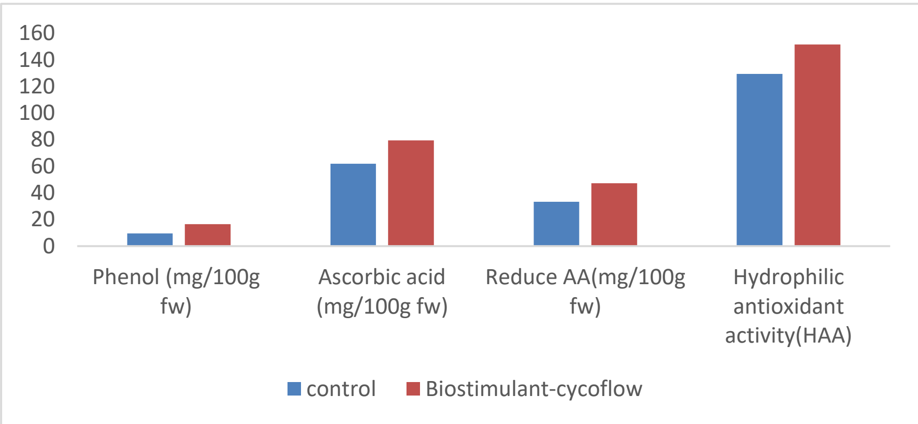

Figure 6. Effect of biostimulant-cycoflow on tomato on heat stress (Source: Francesca *et al*., 2020).

Biostimulants treatments used against heat stress protect cell membranes by increasing their stability and reduce or avoid the accumulation of ROS. Heat stress could negatively impact on the yield by interfering with the reproductive phase, decreasing pollen vitality and germination, suppressing flower differentiation and development and reducing fruit set, which ultimately reduces growth and yield.

Hydrophilic antioxidant activity (HAA), total phenols, and analyses of variance and mean comparison for reduced and total ascorbic acid (AsA), in tomato cultivars treated with the biostimulant CycoFlow administered via fertigation.

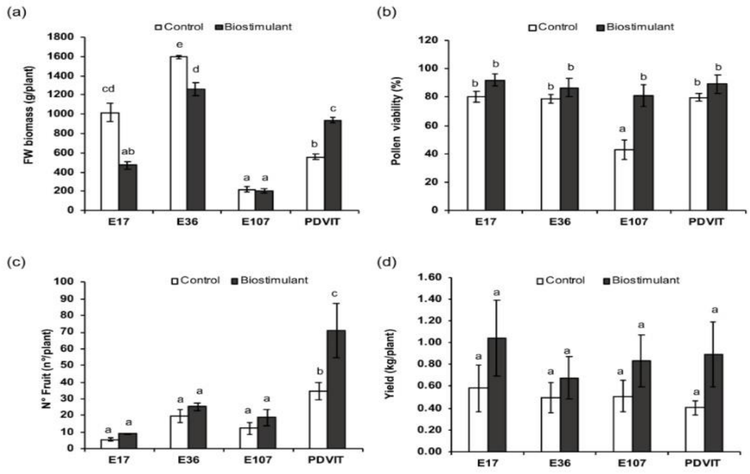

Figure 7. Impact of CycoFlow on four tomato genotypes fresh weight (FW) biomass, pollen viability, fruit quantity, and final yield (a) Fresh weight (FW) biomass, (b) pollen viability, (c) fruit number, and (d) final yield (Source: Francesca *et al*., 2020).

Treated like this. different horticultural crops also showed a high chlorophyll content and improving plant physiological adaptation and improving their growth.

3.3.5 Nutrient Deficiency

One of the roles credited to biostimulant products is the capability to increase nutrient uptake, having low input horticultural production systems (Toscano *et al*., 2013). Under conditions of phosphorus and potassium deficiency, crops treated with the biostimulant exhibited a rise in the growth parameters of shoot length, stem thickness, leaves and roots number, and fresh weight.

In strawberry, the use of alfalfa hydrolysate, vitamins, chitosan, and silicon able to promote biomass accumulation in roots (four to seven folds) and fruits (+20%) of treated plants, whereas the total leaf area increased by 15%–30% and finally total yield performance with fruits effected greatly.

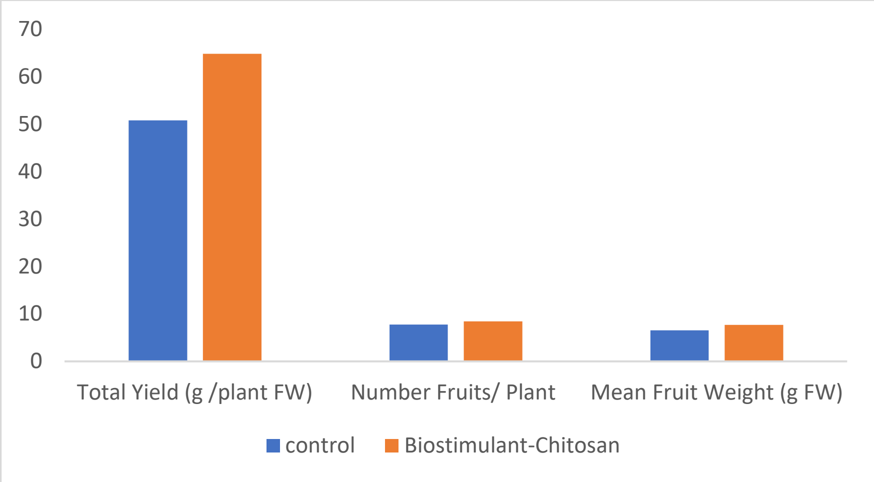

Figure 8. Yield parameters of strawberry as affected by biostimulant products (Source: Soppelsa *et al*., 2019).

Due to the growing role of biostimulants in the horticultural sector, their effect when combined with fertilizers is also of interest. These results may be of interest to growers who want to improve the quality of their ornamental plants by using products that are easy to handle and environmentally friendly.

Table 4. With regard to the percentage of germinated seeds, the test showed that the algae Spirulina and Klamath can significantly improve and also a significant reduction in the average germination time compared to the untreated control.

Groups	seed germination %	average days	Chlorophyl (spad index)
Without algae	88.90	13	19.21
With spirulina	92.30	9	25.08
With Klamath	90.60	11	22.98

(Source: Prisa, 2019)

In the case of early-grown annual ornamental plants e.g. (*Begonia semperflorens*) biostimulants enhance plant outgrowth and increase plant height at the beginning low temperature of cultivation.

3.4 Sustainable Horticultural Production (Fruits, Vegetables) Through Biostimulants Under Abiotic Stress

Biostimulants are a unique tool that might helpful to mitigate from abiotic stress. Biostimulant like seaweed extract can help to mitigate the abiotic stresses of horticultural crops because it releases some beneficial proteins along with osmo-protectants, transporter and detoxifying enzymes. Certain metabolisms can be changed to handle stress by synthesizing regulatory substances such proline, salicylic acid, and abscisic acid (ABA) (Calvo *et al*., 2014). By secreting glycine, it aids in stabilizing the protein structure and cell wall, whereas maintaining turgor pressure prevents the generation of reactive oxygen species (ROS). Likewise, the application of protein hydrolysates like glycine betaine, glutamate, and/or ornithine and arginine can activate the plant defense mechanism which able to protect the plant from the abiotic stress (Calvo *et al*., 2014). In addition, humic substance applications in fruit crops minimize the impact of abiotic stress. Abiotic stressors including salt, dryness, and temperature stress are also combated by chitosan (Qiao *et al*., 2014).

Table 5. Biostimulants efficacy to mitigate different types of abiotic stress in some horticultural crops

Stress	Fruit crop	Biostimulant	Effect	Reference
Cold stress	Grapevines	PGPB	•Promotes Stress-related gene transcripts •↑↑ metabolic activity	Theocharis *et al*., 2012
Heat stress	Apple	Carnauba wax	•↓↓ sunburn damage •↓↓ fruit surface temperature and transmission of ultraviolet radiation	Cowan *et al*., 2016
	Pomegranate	Kaolin	•↓↓ sunburn;fruit cracking and incidence of fruit borer and bacterial blight •↑↑ physico-chemical properties of the fruit	Sharma *et al*., 2018
Drought stress	Citrus	Seaweed extract	•↑↑ growth and stem water potential	Spann *et al*., 2011

	Mango	Potassium silicate	•↑↑ vegetative and productive growth •↑↑ tolerance to water stressed •↓↓ harmful effects of ROS	Helaly *et al*., 2017
	Spinach	Megafol, Aminovert	↑↑nutritional quality	Pereira *et al*., 2019
Salinity	Strawberry	AMF *(Rhizophagus irregularis)*	•↑↑ salt tolerance •↑↑ increased shoot and root mass •Promotes Genotype-specific effect of AMF inoculation	Sinclair *et al*., 2014
	Lettuce	Actiwave	•↑↑ N and Zn content in the leaves	Karapouloutidou *et al*., 2019
	Common bean	Humic acid	•↑↑plant N&P, plant root and shoot dry weight, germination%, biomas chlorophyll, AA	Rady *et al*., 2015
Nutritional defficiency	Apricot	Humic substances	•↑↑ vegetative growth & chlorophyll a •↑↑ leaf N, P, K, Mg content and tree yield	Fatma *et al*., 2015
	Tomato	Megafol	•↑↑ fruit number •↑↑phenol, flavonoid, antioxidant •↑↑proline content	Klokic *et al*., 2020

* ROS=reactive oxygen species; AA=Ascorbic Acid; AMF=Arbuscular mycorrhizal fungi;N-Nitrogen; P=Phosphorous; Zn=Zink; K=Potassium; Mg=Magnesium

Numerous studies on the impact of biostimulants and their use in various horticultural crop species under abiotic stress condition are discussed above. In many fruits, vegetables, ornamentals, herbs, and medicinal plants, various available commercial biostimulants enhance plant stress tolerance and antioxidative capacity, nutrient acquisition and distribution within the plant, growth and development of their vegetative and generative organs.

3.5 Mechanism of Biostimulants

3.5.1 Mechanism of Biostimulants in Crop Stress Mitigation

The mechanisms that biostimulants activate in response to stressful situations are difficult to pinpoint and are still being surveyed. The biostimulants effectiveness to mitigate the stressful condition depends on several factors, such as – timing of application and their mode of action. In general, biostimulants can act on the primary metabolism by increasing photosynthetic activity and derived compounds or they can stimulate the secondary metabolism by activating specific biosynthetic pathways (Bulgari *et al*., 2019; Bulgari *et al*., 2015).

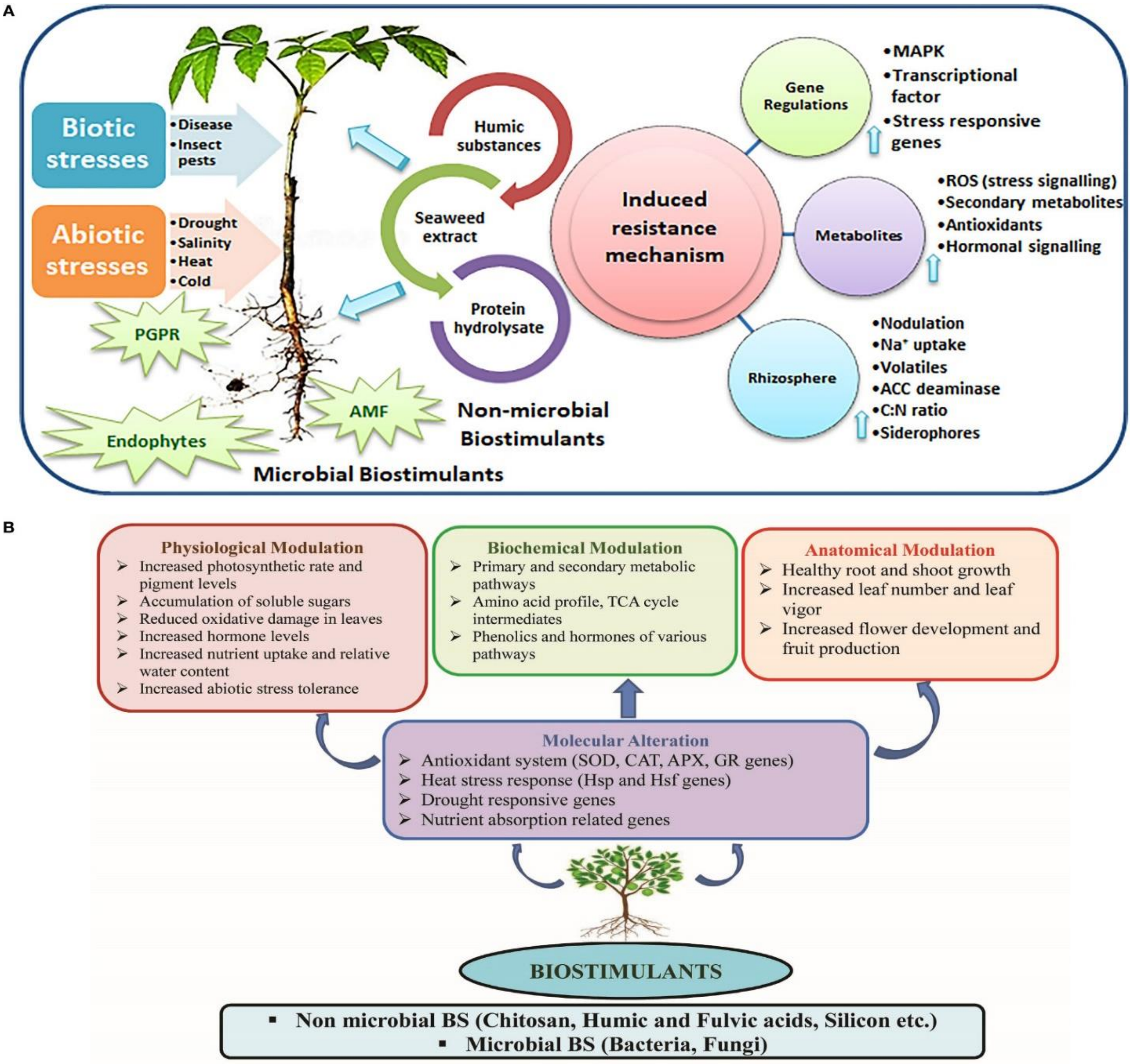

Figure 9. The role of biostimulants in mitigating the adverse effects of stresses (Source: Bhupenchandra *et al*., 2022).

Here in this figure (A, B), action of plants through several mechanisms (molecular alteration, physiological, biochemical, and anatomical modulations) after application of biostimulants are described.

Biostimulants formulations can be used as seed priming agents to anchor synergistic plant growth in the soil, either alone or in combination with micronutrients. They can be applied as liquid foliar treatments or as soil preparations (powders, granules). While several types of plant and seaweed extracts are utilized as foliar sprays, biostimulants with humic substances and nitrogen compounds are frequently applied directly to the soil. (Drobek *et al*., 2019). On the other, those are involved in the process of bioactive compounds biosynthesis must be applied before the stress arrives.

3.5.2 Mechanism of Abiotic Stress Mitigation Through Targeted Different Biostimulants Including Horticultural Crops

Due to their excellent solubility and capacity for multiplication, the application of biostimulants to the horticultural crops could overcome the yield barrier due to environmental stresses. The metabolic pathways that biostimulants have triggered may be enhanced under stressful circumstances, which could help the plants adapt and thereby avoid or delay the most serious problems. There is no general procedure that works for a crop species and in each stress condition. By numerous ways they enhance plant performance, promote plant growth and productivity, interact with several processes attached in plant responses to stress, and increase the accumulation of antioxidant compounds that allow reducing in plant stress sensitivity.

Primary stresses such as drought, salinity, cold, heat and chemical pollution, cause cellular level injury and secondary stresses like osmotic and oxidative stress. The initial stress signals that is osmotic and ionic effects or changes in temperature or membrane fluidity activates the signaling process and transcription panels, which activate stress- mechanisms to restore homeostasis and to defend and repair damaged proteins and membranes (Rana *et al*., 2022).

Seaweed extracts: Seaweeds as biostimulants are emerging as commercial formulations including vast group of macroscopic, multicellular marine algae that can be brown, red, and green. They are an important source of organic matter and fertilizer nutrients and applied as foliar spray as plant growth promoting factors and a method to improve tolerance. Algal extracts focus on a variety of routes to raise stress tolerance.

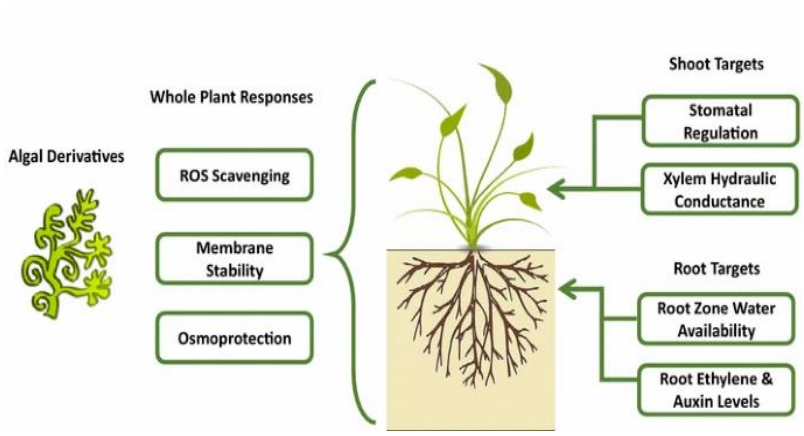

Figure 10. Mechanisms targeted by seaweed(algae)-based biostimulants (Source: Van Oosten *et al*., 2017).

Secondary messengers are activated when the elicitor binds to the algal receptor sites. After cognition of an elicitor, reversible phosphorylation and dephosphorylation of the plasma membrane proteins and cytosolic proteins occur; with activation of the mitogen-activated protein kinase (MAPK) produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) occurs as well as NADPH oxidase initiation. When this occurs, a cascade of chemical reactions in the plant which then allows for resistance and enhanced growth regulation.

Humic substances (HSs): They include humic acids, fulvic acids and humins. It has been seen that in horticultural crops, treatments with humic substances stimulate plants root growth and development (Canellas *et al*., 2002; Trevisan *et al*., 2010). This is reflected in a better uptake of nutrients and water, and enhanced tolerance to environmental stresses.

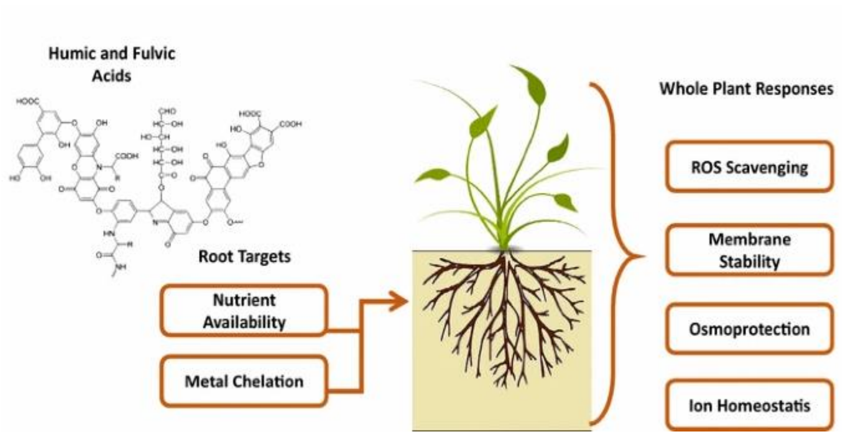

Figure 11. Mechanisms targeted by humic acid-based biostimulants (Source: Van Oosten *et al*., 2017).

Hydrolyzed proteins and amino acids: Protein hydrolysates are mixtures of polypeptides, oligopeptides, and free amino acids derived from partial hydrolysis of by-products from animals and plants may increase stress tolerance through different (Colla *et al*., 2015).

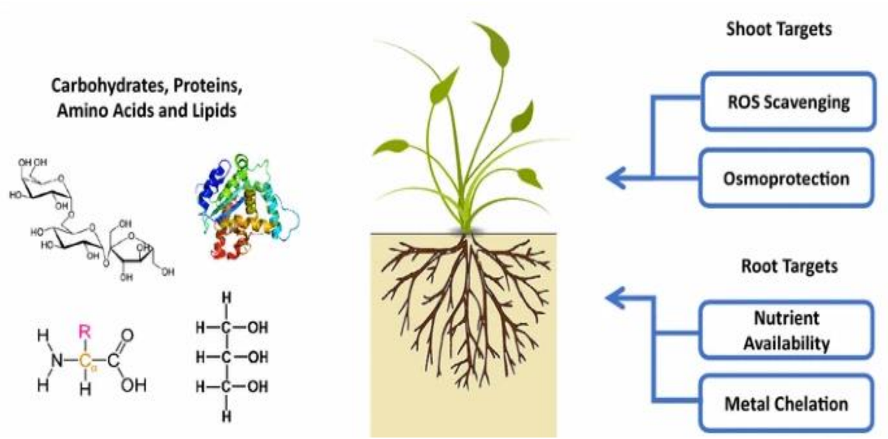

Figure 12. Mechanisms targeted by carbohydrate-, protein-, amino acid-, and lipid-based biostimulants (Source: Van Oosten *et al*., 2017).

Microorganisms: This group having bacteria, yeast, filamentous fungi, and micro-algae. They increase nutrient intake by nitrogen fixation and nutrient solubilization, alter hormonal conditions by changing the production of plant hormones like auxins and cytokines, and also increase resistance to abiotic stressors and generate volatile organic composites (VOCs), which may also have a direct positive-effects on crops by improving nutrient and water uptake.

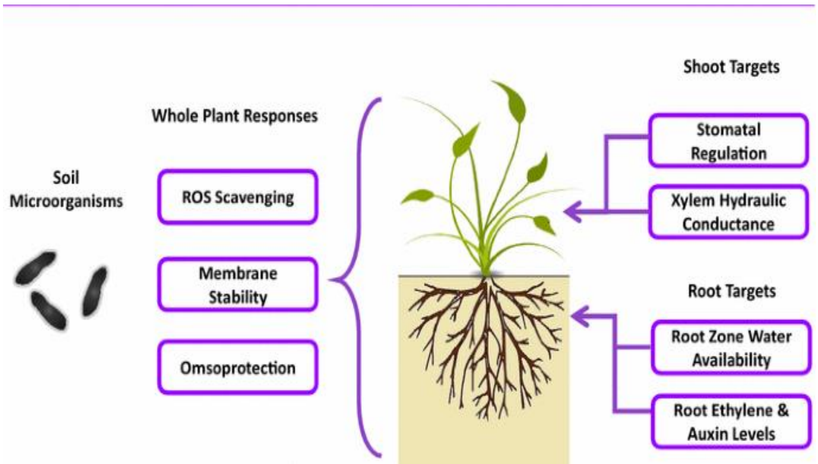

Figure 13. Mechanisms targeted by soil microorganisms-based biostimulants (Source: Van Oosten *et al*., 2017).

Biostimulants can also be made from different compost extracts, manures, vermicompost, aquaculture residues and waste streams, sewage treatment, and extracts of food waste or industrial waste streams (Xu *et al*., 2018). The products, including nanoparticles and nanomaterials, has been recently proposed by Juarez-Maldonado *et al.* (2019). They are able to change the yield's quality and resistance to abiotic stressors, when applied in small amounts as foliar spray or in nutrient solution, and also in vegetable crops (Qi *et al*., 2013; Kiapour *et al*., 2015; Lei *et al*., 2008; Khan *et al*., 2016).

Biostimulants contain anti-stress components, such as proline or glutamic acid that can be applied when the stress comes or during stresses. Megafol is a commercial biostimulant belong to the humic acid group, also seaweed and microbial biostimulants have been largely used for enhanced induction of some drought responsive genes to protect the crops. They show positive effects which generate absorption surfaces in the region of the root systems and isolated soil water in aid of the crops. Biostimulants operation helps sustain cell homeostasis and also prevent environmental and oxidative stress, helps to reduce nutrient deficiencies and improve crops nutrient uptake by increasing root biomass, nutrient transport/translocation, and enzyme activities involved in nutrient assimilation.

CHAPTER IV

CONCLUSION

After considering the aforementioned findings, it can be concluded that biostimulants cannot completely replace fertilizers but could be really useful in different imbalanced situation for improving nutrient uptake and utilization efficiency in crops. They have been used at all stages of horticultural production including as seed treatments, as foliar sprays during growth and on harvested products, having a positive effect on crop growth and protection against several conditions. Mitigate the negative effects of abiotic stresses on crops having marked effects of biostimulants on the control of drought, high heat, salinity, chilling, frost, oxidative, mechanical, and chemical stress.

Furthermore, it has been also noticed that biostimulants both encourage plant development and stimulate the plant's defense mechanism against abiotic stresses. Seaweed extract, humic substance, protein hydrolyzed and amino acids containing products, microorganisms and other compounds can work as biostimulators in horticultural crops to enhance their nutritional quality. In both ideal and unfavorable climatic conditions, they have beneficial impacts on the metabolism of horticultural crops. Therefore, it can be suggested that regular monitoring tools for the efficacy of biostimulants can increasing crop growth performance and yield.

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