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Biostimulants and Its Influence on Soil Properties and Crop Production

VIMAL KUMAR[1*], PRIYANKAR RAHA[2], ANSHUMAN KOHALI[1], CHANDRABHAN PATEL[1] AND VIKAS CHANDRA[3]

ABSTRACT

Environmental pollution is one of the most pressing global issues and it requires priority attention.There is increasing demands to increase the productivity of crops grown in unfavorable soil conditions. Biostimulants are substances widely used in agriculture to raise crop production and resistance to various types of stress and advantages of agricultural practices andit ismore environment eco-friendly products. Theseare new concept has also accepted their ability to work against the injurious effects of pollutants on plants. Biostimulants consist of many different compounds with positive effects on plants, excluding pesticides and chemical fertilizers and regarding various substances of organic compounds. Theyare derived from amino acid hydrolysate, plant extracts biostimulants, humic substances, protein andseaweed extracted are used. The application of biostimulants stimulated microbial activity influences soil biological properties and their microbial communities in field conditions. It also stimulated soil physical properties i.e. soil structure, soil porosity, structural coefficient with increased soil moisture content and decrease bulk density,along with, increase total carbon, nitrogen and the effects of physicochemical properties. These mechanisms of action of biostimulants help to maximize agricultural yields under stress conditions which provoked by climate change. Further, there is need tolong-termstudies to evaluate the effects of biostimulants on crop yield and soil resilience.

Keywords: Agri-horti system, Leaf mulch, Pearl millet

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INTRODUCTION

Green Revolution in order to meet public demand and to sell crop products commercially, applied of chemical inputs like fertilizers, pesticides, and weedicides as well as intensive irrigation practices of green revolution helped achieve the goal to some extent. When crop yields start declining despite fertilizer application following the green revolution, this indicates that the soil has lost its fertility. Beneficial soil organisms can'tliveif there are toxic chemicals in the soil and pollution of groundwater, air as well as bringing hazarads to environment then harms the caused to human and animal health. The first biostimulants product (Maini, 2000) based on amino acids and short chain peptides (Siapton ®,). Theamino acid based biostimulants are now very popular in crop cultivation practice in world. The different commercial products of amino acid biostimulants produce in world market. Amino acids are fundamental ingredients in the process of protein synthesis. About 20 important amino acids are involved in the process of each function. Amino acids can directly or indirectly influence the physiological activities of the plant. Biostimulants can be define as small amounts of organic or inorganic matter that referred to as positive impact of growth regulators or metabolic enhancers and play an important role of soil biological and physicochemical properties that are important to improving and maintaining soil health to achieve sustainable crop production.

These amino acids help to increase chlorophyll concentration in the plant leading to higher degree of photosynthesis and this makes crops lush green. Stomas are the cellular structures that control the hydric balance of the plant, the macro and micronutrient absorption and the absorption of gases. The opening of the stomas is controlled by both external factors (light, humidity, temperature and salt concentration) and internal factors (amino acids concentration, abscisic acid etc). The stomas are closed when light and humidity are low and temperature and salt concentration are high, when stomas are closed photosynthesis and transpiration are reduced (low absorption of macro & micronutrients) and respiration is increased (carbohydrate destruction). In this case the metabolic balance of the plant is negative. Catabolism is higher than anabolism, this implies slow metabolism and stops the plant growth. L-glutamic acid acts as a cytoplasm osmotic agent of the "Guard Cell" and thus favouring opening of the stomas. The constituents of amino acids are varied from one product to other. For example, Siapton contains (Maini,

[1] Department of Soil Science & Agricultural Chemistry (SS&LUPS), BAU, Sabour, Bihar
[2] Department of Soil Science & Agricultural Chemistry, IAS, BHU, Varanasi
[3] Department of Horticulture (Fruit & Fruit Technology), BAU, Sabour, Bihar
*Corresponding Author E-mail: vimal@bausabour.ac.in

2000) a high proportion of proline and glycine while carob germ hydrolysate glutamine and arginine predominate. The Protifert, derived from the collagen protein of animal is composed of glycine (about 30%), proline and hydroxyproline (about 30%).

Materials of biostimulants

The term biostimulants is defined as a substance that is neither a plant nutrient (fertilizer) nor a pesticide, but has a positive impact on plant health. A biostimulants is an organic material (Fig.1) that, when applied in small quantities, enhance plant growth and development such that the response cannot beattributed to application of traditional plant nutrients and these are have no direct action against pests and therefore do not fall within the regulatory framework of pesticides.

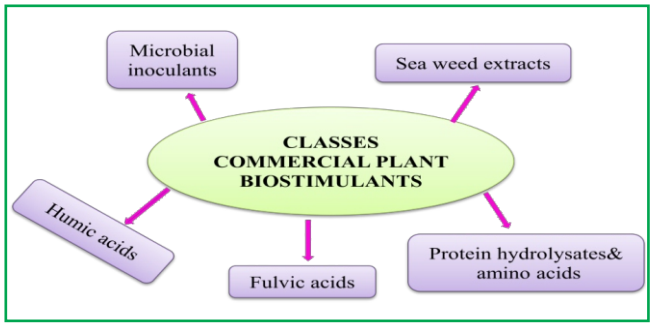
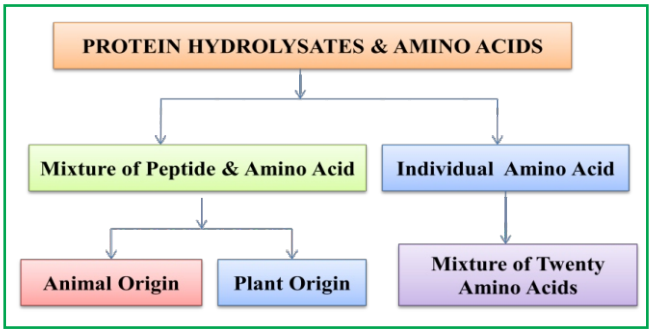


Fig. 1: Origin based of commercial biostimulants

The source of enzymes, proteins and amino acid compounds may be used as biostimulants (Table 1). Natural stimulants are often included under the term biostimulants, including phenols, salicylic acid, humic and fulvic acids, or protein hydrolysis. The protein hydrolysates and amino acids are divided in different originated forms of amino acids biostimulants are;



The mixed amino acids (20/21 amino acids) formulation are also available in market for application of commercial crops. Most of the animal/protein hydrolysate products and mixed amino acid formulation products are organic product accredited by IFOAM (International Federation of Organic Agriculture Movement). Plants absorb amino acids through stomas and are proportional to environment temperature. Amino acids are also supplied to plant by incorporating them

Table 1: Chemical composition of commercial amino acids-based products available in the market.

S. N.	Amino acids	Amino Acid (Per cent)			
		Animal based		Plant origin	
		Free	Total	Free	Total
1	Aspartic acid	0.35	2.25	1.02	1.19
2	Glutamic acid	0.36	7.56	12.2	16.9
3	Phenylalanine	< 0.05	1.21	< 0.05	< 0.05
4	Histidine	< 0.05	0.38	< 0.05	0.15
5	Treonine	< 0.05	< 0.05	< 0.05	< 0.05
6	Arginine	< 0.05	0.38	< 0.05	< 0.05
7	Alanine	2.62	7.57	1.60	1.95
8	Tyrosine	< 0.05	0.13	< 0.05	< 0.05
9	Methionine	< 0.05	0.44	< 0.05	< 0.05
10	Isoleucine	< 0.05	0.77	< 0.05	< 0.05
11	Leucine	< 0.05	2.11	< 0.05	< 0.05
12	Lysine	< 0.05	1.60	< 0.05	< 0.05
13	Serine	< 0.05	0.31	< 0.05	0.52
14	Proline	1.14	6.98	0.088	0.25
15	Cystine	-	0.024	-	0.023
16	Valine	< 0.05	1.29	0.22	0.35
17	Glycine	5.72	14.8	0.14	0.46
18	Hydroxyproline	0.85	4.44	< 0.05	< 0.05
	Total	**11.04**	**52.24**	**15.27**	**21.79**

Source: Cerdan *et al.* (2013)

into the soil. It helps in improving the micro-flora of the soil thereby facilitating the assimilation of nutrients.

Amino Acid Biostimulants for Crop Cultivation

Amino acid biostimulants on the yield and WUE of field experiment of potatocrop was laid out in a three irrigation schedules, I_1 (3 irrigation), I_2 (4 irrigation) and I_3 (5 irrigation) in main-plots and subplotspray three types amino acid based biostimulants, A_0 (control), A1 (animal based), A_2 (plant based) & A_3 (mixture amino acids)) at three application schedules ofamino acid with three replications. The maximum yield ofpotato tuber was recorded both years of experiment were observed in four irrigation schedule and plant based amino acids (I_2A_2) and followed by other treatment. The interaction effect of maximum WUE (water use efficiency) was observed in I_1A_3 treatment combination in the year 2014-15 and I_2A_2 treatment combination in the year 2015-16 experiment of potato crop (Kumar *et al*., 2018).

Amino acids have a chelating effect on micronutrients. When applied together with micronutrients, the absorption and transportation of micronutrients inside the plant is easier. This effect is due to the chelating action and to the effect of cell membrane permeability. L-glycine & L-glutamic acid is known to be very effective chelating agents. The effect of foliar spray of certain Zn-amino acid chelates, including Zn-arginine, Zn-glycine and Zn-histidine on yield and grain quality of two different Zn-deficiency tolerant wheat cultivars (*Triticum aestivum* cvs 'Back Cross' and 'Kavir') was (Ghasemi *et al*., 2013) under field conditions. Foliar application of Zn, regardless of the used source improved grain yield of both wheat cultivars. Grain Zn, iron (Fe) and protein concentrations higher in wheat plants sprayed with Zn-amino acid chelates than those sprayed with $ZnSO_4$.The significant positive correlation between grains Zn, Fe, and protein concentrations indicates that the genes affecting the grain accumulations of Zn, Fe and protein are probably closely linked. Foliar application of Zn fertilizers resulted in significant decrease of grain phytic acid (PA) and PA: Zn molar ratio in comparison with the control treatment although the magnitude of this reduction was greater for Zn-amino acid chelates than $ZnSO_4$. The Zn-amino acid chelates should be considered as new Zn fertilizer sources for improving yield and bioavailable Zn concentrations of wheat grain.

The effect of some biostimulants i.e. ascorbic acid, dry yeast, amino acids (Ruter and Total), seaweed extract and Spirulina extract (*Arthospirafusiformis*) on plant growth, yield (Shalaby and EI-Ramady, 2014) components and storability of garlic plants "Balady" cultivar. The effect of foliar application of Amino Total increased plant height compared to all treatments and control and yeast or amino Total amino acid showed the heaviest bulb weight.

The multiple functions of Siapton (Maini, 2000) such as a nutritional and a general biostimulants effect. The products have been used by Italian and European farmers were found on many crops such as sugar beet, potato, strawberry, cereals, citrus, horticultural crops, tomato, grapevine, fruits, cotton, olive, mushrooms, etc and the results were always positive as for as the quantity and quality are concerned. Also, the main mechanisms were demonstred; (i) positive action on some enzyme systems (among them: Nitrate Reductase, GDH-NAD dependent; Malate dehydrogenase, Leucino-amino peptidase, Phosphorilase, Phosphatase). (ii) Strong effect toward biotic and abiotic stress due to some enzymes systems, as well as the high content of proline and hydroxyproline. (iii) Positive action on the endogenous and exogenous plant growth regulators and on the flower fertility and fruit setting.(iv) Indirect biostimulants mechanism due to the chelate and complex formation with the main nutrients and microelements.

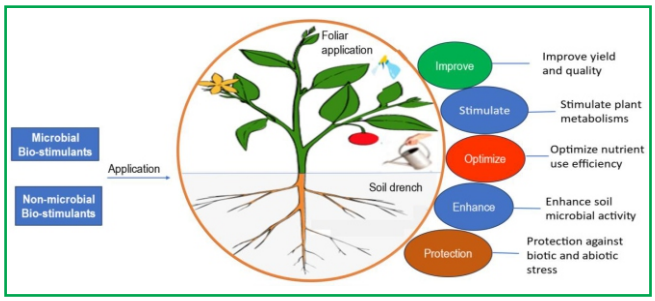


Fig. 2: Biostimulants effect on different aspects of plant growth and productivity by **Rajput *et al*. (2019)**

Table 2: Biochemical action of individual amino acids based biostimulants in plant.

Amino acid	Biochemical action
Glycine	Precursor of chlorophyll.
Proline & Hydroxyproline	Regulators of the water equilibrium (strong antistress &antisenesescence effect),
	Formation of the cell walls (nematostatic action).
	Essential to form fertile pollen in flowers (better fruit-setting).
Glutamic acid & Glutamine	Organic nitrogen reserve to form other amino acids and proteins through transaminase reactions.
Serine	Regulator of water equilibrium, very important for the chlorophyll synthesis.
Arginine	Precursor of polyamines: very important to start the cell multiplication.
Phenylalanine	Precursor of the formation of the lignin, formation of the wooden tissues and stronger defence.
Alanine	Plays a very important role in the hormone metabolism and in the mechanism of virus.
Tryptophan	Precursor of indol-acetic acid, the natural auxine PGR.

Organic biostimulants such as protein hydrolysates, humates are becoming popular, as they reduce the use of chemical fertilizer up to 50%. The increasing effect of protein hydrolysate on plant growth and yield across crop species in both soil and foliar treatments had a higher impact on initial plant growth and the soil application was more effective than foliar application. All parameters recorded (Fig. 2) including root and shoot length, leaf area, total chlorophyll content, photosynthetic rate, and yield were greater in treated plants. The protein hydrolysate when supplemented through soil, influences plant growth and metabolism; thereby, contributing towards higher crop yields. Hence, protein hydrolysates can be effectively used as organic fertilizers to improve productivity of the crops.

The field (Karthikeyan and Shanmugam, 2016) application of commercially manufactured biostimulants (Brand name: AquaSap) from seaweed (*Kappaphycusalvarezii*) sources of numbers of amino acids. Efficiency of biostimulants was tested at 5% through foliar application in selected important vegetable crops 3 to 4 applications were applied based on the crop cycle of the plant. Vegetable crops (tomato, okra, chillies, capsicum, ash gourd, pumpkin, snake gourd, ridge gourd, bottle gourd, bitter gourd, cucumber, watermelon, cabbage, cauliflower, beetroot, carrot, radish, lima bean, soyabean, moringa, onion, potato etc.) were increasing response towards biostimulants applied in terms of general health of the plant, growth, yield and quality of the vegetable production improved quality in all 27 vegetable crops. Therefore, seaweed biostimulants will have enormous potential to organic vegetable production in future.

Amino Acid Biostimulants on Physical Property of Soil

The application of two types of commercially available biostimulants are; Universal Natural Plant food and Convert Seed Primer. The effect of biostimulants had a positive effect on soil moisture content and water holding capacity in all sites and improved some soil biological and physicochemical properties (Wadduwage *et al.*, 2023) that are important to maintain soil health. Dominant bacterial and fungal populations under biostimulants treatments were previously reported as plant growth promoting microbes and efficient decomposers of SOM and shift microbial community composition that was linked to key soil processes and these process impacts on some soil physico-chemical properties. The bioactive elements are biostimulants elucidating underlying biochemical, physiological and molecular pathways of biostimulation. Biostimulants stimulate microbe-derived hormones in root microbiome assembly, rhizosphere, entry to plant vascular system, and root-shoot signaling. The recent advancements in omics-based and other technologies, such as meta-transcriptomics, meta-proteomics or metabolomics, amplicon sequencing and phenotyping, will contribute to profiling of trace metabolites facilitating the soil-microbe-root-shoot processes, and consequently help assess plant performance and yield, quality, and resilience consistently in organic agriculture.

Biostimulants are enhancing soil buffering capacity, stability of aggregates and specific surface area (Papnai*et al.*, 2022). The use of environmentally friendly natural preparations is especially significant in light of the ongoing processes of soil degradation and air pollution. Even while a biostimulants may not have a short-term effect, ithas the potential to improve soil health (Fig. 3) with progression, ensuing higher yields in the succeeding years. The impact of biostimulantson soil physical property in terms of the yields of wheat (spring and winter) and winter rape crop. Numerous soil

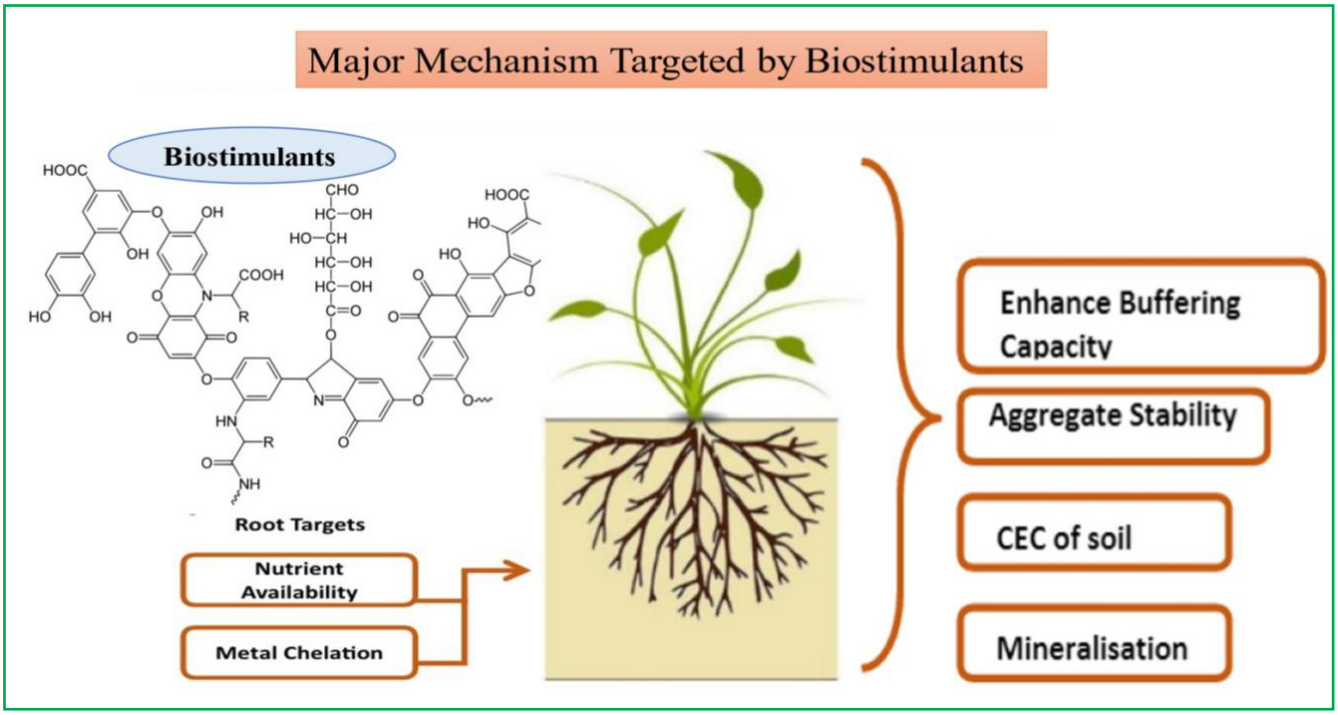


Fig. 3: Effect of Biostimulants on properties of soil quality by **Papnai *et al.* (2022)**

characteristics related to soil properties evaluated at i.e. bulk density, soil porosity and structural coefficient. The impact of application of biostimulantshas increase effect on porosity, structural coefficient, yields of crops and decrease bulk density of soil.

Impact of Biostimulants on Soil Enzyme Activity

Equilibrium of the microbial flora of the agriculture soil is a basic question for a good mineralization of the organic matter and also for a good soil structure and fertility around the roots. L-methionine is precursor growth factors that stabilize the cell walls of the microbial flora.The soil enzyme activities (dehydrogenase, protease and alkaline phosphatase) were significantly enhanced by the spraying (Kumar *et al*. 2018) of all the three amino acids based biostimulants on crop and the order of overall impact of soil enzyme activities in soil due to application of amino acids biostimulants was plant based>animal based>mixture amino acids. Thus, amino acids application improved the soil health plant growth and development.

Thepotential effects of organic biostimulants on soil activity and atrazine biodegradation in Carob germ enzymatic extract and wheat condensed distiller solubles enzymatic extract (García-Martínez, 2010) have been obtained using an enzymatic process; their main organic components are soluble carbohydrates and proteins in the form of peptides and free amino acids. Their application to soil results in high biostimulation, rapidly increased dehydrogenase, phosphatase and glucosidase activities, and an observed atrazine extender capacity due to inhibition of its mineralization. The extender capacity of both extracts is proportional to the protein/carbohydrate ratio content. As a result, these enzymatic extracts are highly microbially available, leading to two independent phenomena, fertility and an atrazine persistence that is linked to increased soil activity.

Amino Acid Biostimulants on Nutrient Uptake Efficiency

The corresponding uptake and assimilation of NO_3^- in roots and leaves by the application of mixed amino acids in red pepper. Nitrate uptake was determined by following NO_3^- depletion from the uptake solution. The activity of the enzymes related to the process of NO_3^- reduction and the content of NO_2^-, NO_3^-, and the end-products (Fig. 4) of this process (amino acids and proteins).

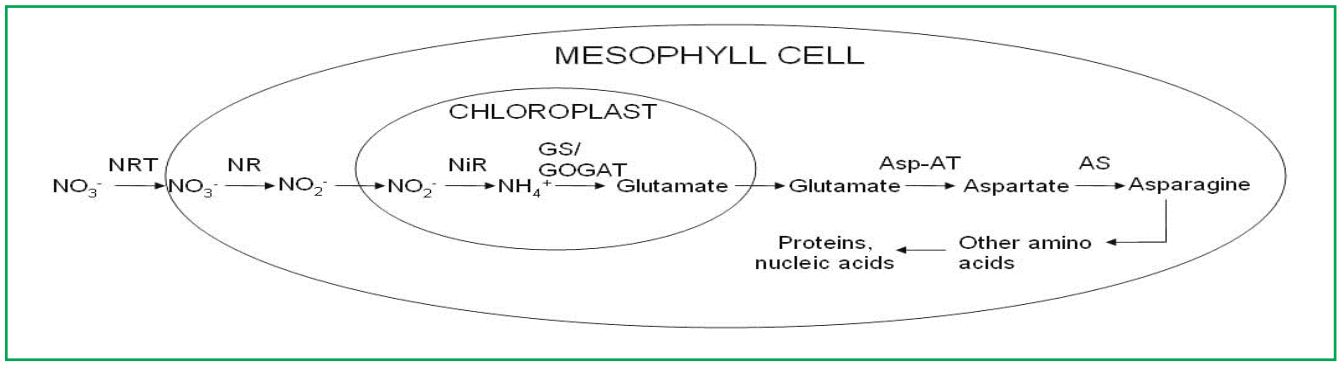


Fig. 4: Process of uptake and assimilation of nitrate

Nitrate uptake was determined (Liu *et al*., 2012) by following NO_3^- depletion from the uptake solution. The activity of the enzymes related to the process of NO_3^- reduction (NR: nitrate reductase; NiR: nitrite reductase; GS: glutamine synthetase) and the content of NO_2^- and NO_3^- were analyzed in shoot and roots. The ability of individual amino acids to regulate nitrate uptake. Glutamine was readily absorbed by the cells and was particularly effective in supporting optimum cell growth in the absence of an inorganic nitrogen source as compared to the three other amino acids evaluated. However, neither glutamine nor any of the remaining 19 protein amino acids appeared to be solely responsible for regulation of nitrate uptake and induction. The ability of amino acids to regulate nitrate uptake and assimilation appears to be more related to their overall levels in the cell rather than to an accumulation of a specific amino acid. The uptake of nitrogen (N) by roots is known to change with supply in a manner that suggests that the N status of plants is somehow sensed and can feedback to regulate this process. The most abundant source of N in soils for crops is nitrate. Uptake systems for nitrate, ammonium, and amino acids are present in the roots of most plants including crops. As nitrate is assimilated via conversion to nitrite, then ammonium into amino acids, it has been suggested that the internal pools of amino acids within plants may indicate nitrogen status by providing a signal that can regulate nitrate uptake by the plant. The role of mixed amino acids in nitrate uptake and assimilation was evaluated in leafy radish. The mixtures of alanine, β- alanine, aspartic acid, asparagines, glutamic acid, glutamine, and glycine were sprayed to plant leaf two or four times. The activity of the enzymes related to the process of NO_3^- reduction (nitrate reductase, nitrite reductase and glutamine synthetase) was affected differently depending on the application rate of mixed amino acids.

The supply of high-quality produce of green bean investigated the efficiency of using micronutrients or amino acids mixed with some micronutrients to improve growth and production of green bean (*Phaseolus vulgaris*, L.) improved growth parameters i.e. plant height, number of leaves and fresh and dry weights was affected. Pod yield was positively correlated with the applied concentration of the two substances with the highest and Pod quality particularly

protein contents responded more positively to Amino-green application while fiber contents responded negatively to all applied treatments. The sprays of Oligo-x, commercial product of algal extract (Abbas, 2013) and the extract contain the protein and amino acids. Field bean plants at determine the growth parameters, phytohormones, pigments, total carbohydrate, protein and minerals. The addition of biostimulants increased pigments, carotenoids concent-rations, total carbohydrates and proteins. The increase quality of bean plants and when are used for organic farming, can reduce our dependence on chemical fertilizers.

Amino Acid Biostimulants on Stress Resistance

The effect of abiotic stress such as high temperature, low humidity, frost, pest attack, hailstorm, floods have a negative effect on plants metabolism with a corresponding reduction in crop quality and quantity. The application of amino acids before, during and after the stress conditions supplies the plants, with amino acids which have directly related to stress physiology and thus has a preventing and recovering effect.The effect of biostimulants promotes plant growth under abiotic stress (Fig. 5) and improves crop productivity without negative impact on the environment (García-García *et al*., 2020).

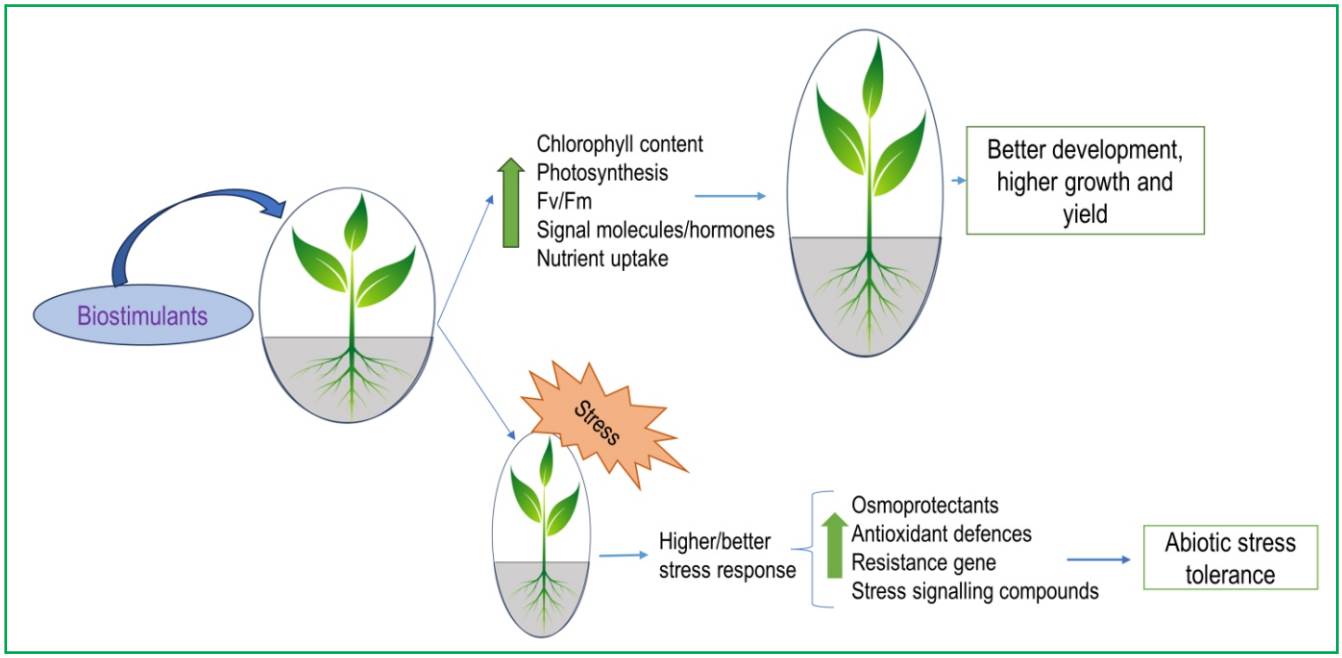


Fig. 5: Effect of biostimulants on plant stress by García-García *et al.* (2020)

The effect of Siapton on growth, water status and primary metabolic changes caused by moderate salt stress with young maize plants grown hydroponically in a growth chamber. The anti-stress action of Siapton is attributed to its specific composition as a balanced mixture of free and short-chain peptide-bond amino acids. The beneficial effects of Siapton demonstrated at all studied level suggested that this ecological product can interfere in the osmostress primary metabolic changes as a non-specific stress mitigation bio-regulator.

The biostimulants application asSiapton®, an amino acidic solution derived from hydrolysed animal proteins is a well-knownbiostimulants which significantly mitigates the damages caused by abiotic stresses (salinity, pollutions, extreme temperatures, drought, etc.) if applied either on the leaves or roots. For example, root application of Siapton on plants grown in high salinity soil com-pletely rescues the growth inhibition caused by the salt. Siapton produces also an effect of normalization of the physiological parameters (contents of potassium, sodium, copper, zinc and iron in vegetal tissues; stomatic conductance; CO_2 absorbance; transpiration) usually altered in all the untreated plants.Drought is one of the most significant abiotic stresses that limits the growth and productivity of crop plants. The physiological and molecular responses of tomato plants treated with Megafol® (Valagro S.P.A) under specific drought conditions (Petrozza *et al.* 2014). The goal was to evaluate the impact of Megafol® a biostimulants composed of a complex of vitamins, aminoacids, proteins and betaines in attenuating the negative physiological responses of drought. The effect of Megafol® were healthier in terms of the biomass produced and chlorophyll fluorescence, thus highlighting the higher tolerance to stress of the treated plants andthe Megafol® were also studied at a molecular level by analyzing the induction of genes typically involved in drought stress responses. Our results demonstrate the efficacy of Megafol® to reduce drought-stress related damage in tomato plants.

CONCLUSION

Organic source of biostimulants play an important role of enhance the facilitates the different metabolic processes of crop such as protein synthesis, stress resistance, photosynthetic activities, action of stomas, chelating of micronutrients, decrease the nutrient-leaching losses,

pollination and fruit formation of plant. The glycine and glutamic acid are fundamental metabolites in the process of formation of vegetable tissue and chlorophyll synthesis to deliver enhanced plant growth, yield, quality, and resilience consistently in organic agriculture.Themicrobial activities efficient decomposers of SOM and can shift microbial community composition that were linked to key soil processes and long-term impact of PGPR on soil health which plants utilize soluble nutrients. Soil health is improved, as is agricultural resilience to climate shocks. It is reasonable to assume that biostimulants will play an increasingly important role in the development of environmental and economical viable crop production systems within more resourceful agro-ecosystems.

Future prospectus

A globally demand for organically grown crops healthier and safer food products. From a sustainability perspective, organic farming offers an eco-friendly cultivation system that minimizes agrochemicals and known to help plants cope with stressful situations like drought, salinity, extreme temperatures and even certain diseases. Compatible with organic farming, the selective use of biostimulants can close the apparent yield gap between organic and conventional cultivation systems. The organic base productive of bioactive sub-stances as biostimulants from agro-industrial products and allows carrying out a better cleaning of polluted environments. The use of biostimulants should improve plant growth and resilience to biotic and abiotic stresses and deliver acceptable yield and good quality organically cultivated products and economical viable crop production systems within more resourceful agro-ecosystems. The ground work for a bio-based industry challenging need for researchers and fertilizer manufacturers to better a lot of big players in the agro-industry who see plant biostimulants as a legitimateagri-input and a good business idea. The future demand of this study was to evaluate the potential of biostimulants to improve soil properties and crop yields. Products like biostimulants are seen as an entirely new generation of agricultural tools for sustainable farming.

Conflict of Interest

The Authors declare that there is no conflict of interest among the authors.

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