

The influence of biostimulants on the growth and on the biochemical composition of *Vicia faba* CV. Giza 3 beans

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Abstract

The experiment was conducted in a glass house at the Faculty of Education, Ain Shams University. Sprays were carried out at intervals of 15 days each were given during vegetative growth. Field bean plants at 30 days from sowing were sprayed with the following treatments -Water (Control), Bacillus licheniformis (10^{10} cells/ml), Yeast (5 g/L), Algal extract (0.1%) and Humic acid (20 g/L). The plants were collected after 90 days from sowing to determine the growth parameters, phytohormones, pigments, total carbohydrate, protein and minerals. The growths of plants were increased except in number of leaves of the algal extract treated plants. On the other hand, these treatments significantly increased auxins, and gibberellins in treating plant shoot, while abscisic acid was decreased. The addition of biostimulants significantly increased pigments, carotenoids concentrations and total carbohydrates. Biostimulants increased proteins significantly except in bacteria treatment plants where the protein increase was insignificant compared with control. Magnesium, nitrogen and phosphorus significantly increased, while calcium was decreased by the algae extraction compared with untreated and treated plants. The aim of the present study can be recommended in agriculture to increase quality of bean plants and when are used for organic farming, can reduce our dependence on chemical fertilizers.

Keywords: Biostimulants, Chemical Composition, Growth, Field Bean

Introduction

Biostimulants are an organic material that has been shown to influence several metabolic processes such as respiration, photosynthesis, nucleic acid synthesis and ion uptake and when applied in small quantities, enhances plant growth and development. They are mixtures of one or more things such as microorganisms, yeast, seaweed extracts, plant growth promoting rhizobacteria (PGPR) and humic acid trace elements, enzymes and plant hormones. They may enhance water-holding capacity, increase antioxidants, and enhance metabolism [23]. Humic acids have been shown to stimulate plant growth and yield by acting on the mechanisms involved in: cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities, improvement of soil structure and increase of microbial populations. Algae extract as a new bio fertilizer containing N, P, K, Ca, Mg, and S as well as Zn, Fe, Mn, Cu, Mo, and Co, some growth regulators, polyamines, natural enzymes carbohydrates, proteins and vitamins applied to improve vegetative growth and yield [2]. Active dry yeast is a natural safety biofertilizer, it is considered as a natural source of cytokinins that stimulates cell division and enlargement as well as the synthesis of protein, nucleic acid. Bacteria are able to exert positive effects on plants through several mechanisms; maintaining soil fertility, nitrogen fixation, promoting root elongation, solubilize insoluble minerals through the production of acids, increasing the availability of phosphorus and other nutrients in soil [16]. Therefore, N₂-fixing and phosphate solubilizing bacteria, including *Bacillus* sp. are widely used in organic plant growing [15]. The aim of the present study was to evaluate the effects of these biostimulants on plant growth of the tested plants.

Materials and Methods

The bean plants (*Vicia faba* CV. Giza 3 modified) is a class hybridization breeder, bear shoot disease [spotting brown and rust. Largesse grown in the provinces of Lower Egypt. Flora strong growth medium branching . Seeds of field bean plants (*Vicia faba* CV. Giza 3 modified) were sown during the growth season in pots containing 3.5 kg of homogeneous loamy clay soil. The pots for each treatment were replicated . Field bean plants at 30 days from sowing were sprayed with biostimulant treatments and Seedlings inoculated with *Bacillus licheniformis* (10^{10} cells/ml). All of the treatments used to be in liquid form (100 ml was applied using distilled water). The pots were irrigated with the water holding capacity of the soil (80%). The bacterial strains (*Bacillus licheniformis*) were grown in the appropriate medium [21], incubated at 28°C for 5 days, and diluted to a final concentration of (10^{10} cells/ml) in sterile distilled water. Seedlings inoculated with *Bacillus licheniformis* (10^{10} cells/ml) was done twice, at after 15 days of planting and after 30 days of planting. Active dry yeast was dissolved in water followed by adding sucrose at ratio 1:1 and kept overnight to activate and sprayed on the plants. The chemical composition of bread yeast is presented in Table 1 by means of Nagodawithana [18].

Algae extract (oligo-x) was obtained from (Arabian group for agricultural service) company having the following composition: oligosaccharide (3%), algnic acid (5%), natural growth regulators (cytokinine, 0.001% ; indole acetic acid, 0.0002% and pepsin, 0.02%) and minerals (potassium oxide, 12% ; phosphorus oxide, 0.5% ; N, 1% ; Zn, 0.3% ; Fe, 0.2% and Mn, 0.1%). Humic acid, (Hammer, Arabian Group for Agriculture Service AGAS Company). Hammer composition (humate potassium 86% and potassium oxide 6%). At 90 days from sowing, the 10 plant samples for each triplicate were collected to determine the growth parameters and phytohormones, photosynthetic pigments, total carbohydrate content, total protein and mineral contents

Table 1. Chemical composition of bread yeast (6)

Lipids4%	Approximate composition of vitamins (mg/g):		Approximate composition of minerals (mg/g):			
	Thiamine6-100	Biotin 1.3	Na 0.12	Cu 8.00	K 21.00	Ni 3.00
Protein 47%	Riboflavin35-50	Cholin 4000	Ca 0.75	Se 0.10	P 13.50	Mo 0.40
Carbohydrates 33%	Niacin300-500	Folic acid 5-13	Fe 0.02	Mn 0.02	S 3.90	Li 0.17
Minerals8%	Pyridoxine 28	Vit-B12 0.001	Mg 1.65	Cr 2.20	Zn 0.17	Si 0.03
	Pantothenate 70					

The growth parameters of field bean plants were obtained by drying the material in oven at 80°C to constant weight. The sample of field bean, plant shoots were taken from each treatment frozen with liquid air and stored at - 200C for estimating the endogenous hormones according to the method described by Badr et al. [18] then the samples were estimated by high performance liquid chromatography (HPLC).

A weight (0.5 g) of fresh young field bean leaves was homogenized in 85% acetone using for determination of photosynthetic pigments as a spectrophotometric method developed by Metzner et al. [17].

Total soluble carbohydrates were determined as methodical of Homme et al. [12].

The total soluble protein content in the supernatant was determined according to the method of Bradford [7,13].

Potassium, calcium, nitrogen and magnesium were determined according to Allen et al. [15]. Flame photometer was using for potassium determination, while atomic-absorption spectrophotometer was used for determination of calcium and nitrogen and magnesium determination. In addition, phosphorous was determined according to Cooper [17]. The collected data were subject to statistical analysis of variance using the normal (F) test and the means separation were compared by using Least Significant Difference (LSD) at the 5% level according to Snedecor and Cochran [23].

Results and Discussion

Growth parameters:

All the studied growth characters of shoots and roots of field bean plants significantly increased except in number of leaves of the algal extract treated plants comparing with control (Table 2).

The beneficial effect of humic acid on plant growth may be attributed to the promoting effects on nutrient uptake and nutritional status especially nitrogen, potassium and phosphorous necessary for plant growth [10]. In addition, it is acting as a source of plant growth hormones, carbohydrates, amino acids and vitamins. The algae extraction effects on seedlings may refer to algae extraction contains the nutrient elements and growth hormones that increased the seedling growth [2]. The incremental effect of yeast extract might attribute to the influence on metabolism and their stimulating effect on photosynthetic pigments and enzyme activity that in turn increased vegetative growth of field bean. Plant growth promoting rhizobacteria (PGPR) is able to exert a beneficial effect on plant growth due to the addition of PGPR increase germination rate, root growth, yield, and leaf area [12].

Table 2. Effects of bacteria, yeast, algal extract and humic acid on some growth parameters of field *Vicia faba* CV. Giza 3 beans

Treatment	Shoot length (Cm)	Root length (Cm)	Stem diameter (mm)	No. of leaves	Fresh Weight of roots (g)	Dry Weight of roots (g)	Fresh Weight of shoots (g)	Dry Weight of shoots (g)
Control	41.6 ± 1.65	19.80 ± 0.96	3.58 ± 0.41	15.20 ± 0.65	5.09 ± 0.41	0.38 ± 0.02	11.16 ± 0.36	0.89 ± 0.05
<i>Bacillus licheniformis</i>	43.6 ** ± 1.42	33.60 ** ± 0.85	4.00** ± 0.32	15.4** ± 0.72	5.35** ± 0.36	0.49** ± 0.04	13.28** ± 0.28	1.00** ± 0.04
Yeast	42.8 ** ± 1.31	26.20 ** ± 0.91	4.50** ± 0.50	16.40** ± 0.54	6.50** ± 0.27	0.63** ± 0.03	16.34** ± 0.43	1.22** ± 0.03
Algal extract	42.4** ± 0.96	25.80** ± 0.76	4.52** ± 0.46	13.40** ± 0.81	5.12 ± 0.33	0.40** ± 0.01	15.84** ± 0.19	1.13** ± 0.04
Humic acid	45.8** ± 1.58	30.2** ± 0.65	3.87** ± 0.29	15.60** ± 0.63	6.54** ± 0.57	0.52** ± 0.05	15.23** ± 0.45	1.20** ± 0.06
L.S.D. at 5%	0.23	0.74	0.06	0.16	0.11	0.01	0.31	0.02

*Highly significant change.

Endogenous phytohormones:

As regards the endogenous phytohormones, the obtained results showed that the different applied treatments increased the levels of endogenous indole acetic acid (IAA), and gibberellins (GA3) in treating bean plant shoots as compared to control, while abscisic acid (ABA) content was decreased Figure 1.

Biostimulants can alter the hormonal status of a plant and exert influence over its growth. The results that were obtained may be due to the potential biostimulant effects as are hormonal in nature by providing plant hormones, enhancing hormonal activity direct supply of plant hormones [23]. Humic contain a gibberellins and auxin, provide the plant with hormones such as auxin and cytokinins and inhibit IAA- oxidase, thus prevents destruction of this plant growth hormone [10]. Plant responses to seaweed extract have been greater than when purified cytokinin was applied alone, they used as a plant growth regulator on plants [23]. Yeast treatments showed the highest values of cytokinins, IAA and GA3 of leaves [21]. *Bacillus* spp. have the ability to alter phytohormone levels can affect the plant's hormonal balance by the gene encoding the enzyme responsible for the synthesis of phytohormones [16]. Plant-growth-promoting effects of *Bacillus pumilus* strains are induced by its capabilities to produce gibberellins.

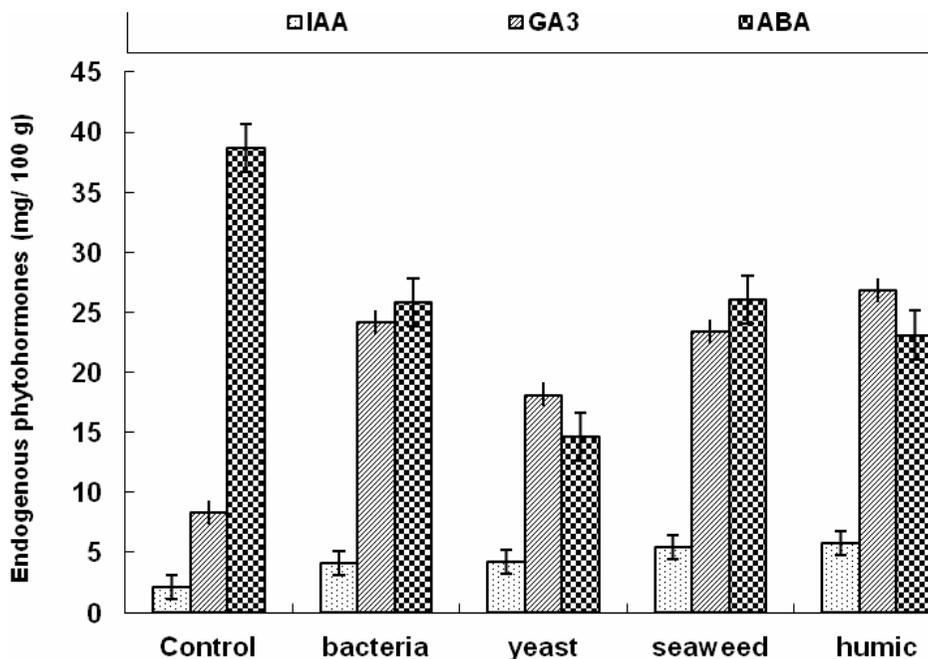


Figure 1. Effects of bacteria, yeast, algal extract and humic acid on endogenous phytohormones of field *Vicia faba* CV. Giza 3 beans

Photosynthetic Pigments:

The results in (Figure 2) indicated that addition of different biostimulants significantly increased pigment concentrations in leaves of field bean. Leaf chlorophyll content with bacteria treatment was insignificant compared with values of algae extraction treatment. The important role of biostimulants on enhancing the leaf chlorophyll might be attributed to their action on increasing the availability of water and minerals the high chlorophyll content might have resulted from enhanced plant growth [16]. Sargassum extract was effective in enhancing chlorophyll synthesis in *Zea mays* and *Phaseolus mungo* [12]. Applying yeast to field bean plants increased contents of chlorophyll a, b, and total chlorophyll [12].

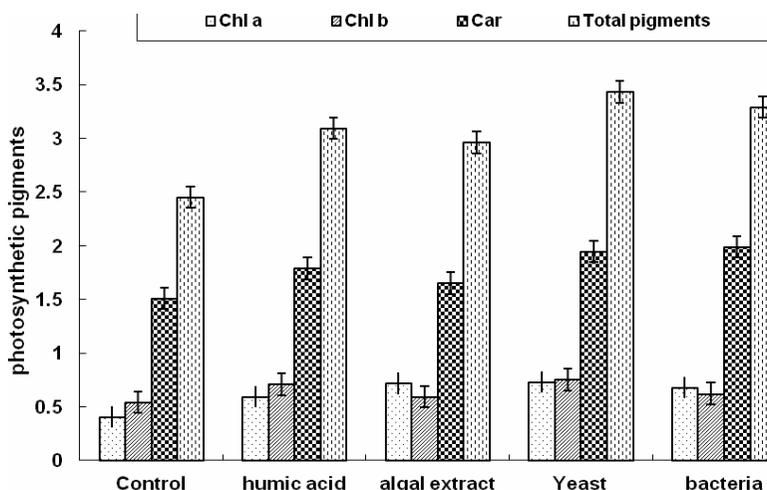


Figure 2. Effects of bacteria, yeast, algal extract and humic acid on photosynthetic Pigments contents of field *Vicia faba* CV. Giza 3 beans

Total carbohydrates:

The results in (Figure 3) showed clearly that all treatments caused significant increase in total carbohydrate contents as compared with control. The best results were obtained from bacteria inoculation treatment.

The concentration of total soluble sugars and reducing sugars were increased at higher concentrations of seaweed extracts [16]. Yeast extract gave the best values of total sugar content and its beneficial effect on carbohydrate accumulation in leaves of field bean [16]. Some studies involving plant growth promoting rhizobacteria inoculation stimulated carbohydrate biosynthesis.

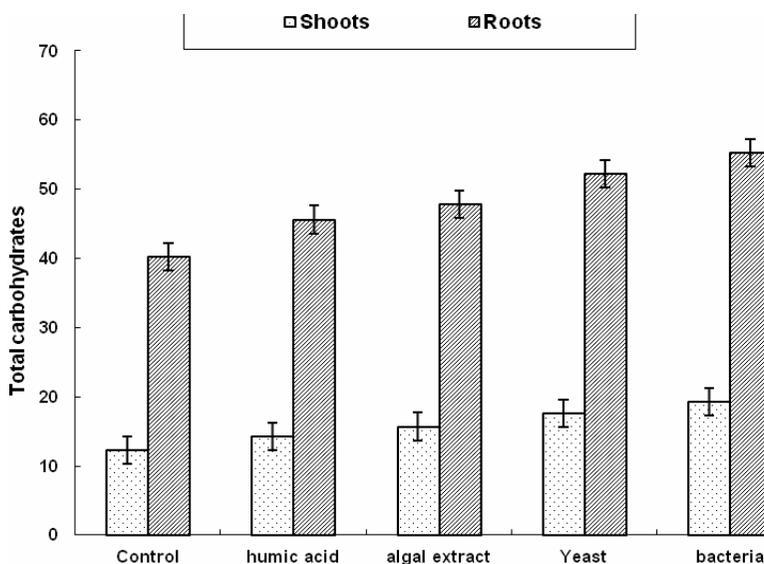


Figure 3. Effects of bacteria, yeast, algal extract and humic acid on total carbohydrates n contents of field *Vicia faba* CV. Giza 3 beans

Total protein:

Applications of biostimulants were significantly increased total protein concentrations expect in bacteria treatment was insignificant compared with control (Figure 4).

The effects of humic acid on increasing some organic compounds as total free amino acids due to it is effect on increasing the uptake of nitrogen to the plant that is a precursor of amino acids [13]. The application of seaweed extract resulted in a significant increase in protein concentration; this due to by either increasing protein biosynthesis or decreasing its oxidation [2]. The increase in protein percentage and free amino acid could be attributing to the growth hormones produced by yeast or due to that yeast, application stimulate the synthesis of protein [21]. Yeast as a natural source of cytokinins-stimulates cell division and enlargement as well as the synthesis and enlargement as well as the synthesis of protein, nucleic acid [16]. The mechanisms by which PGPR promote plant growth are thought to their ability to produce plant hormones that can modulate plant growth and development by nitrogen fixation [22].

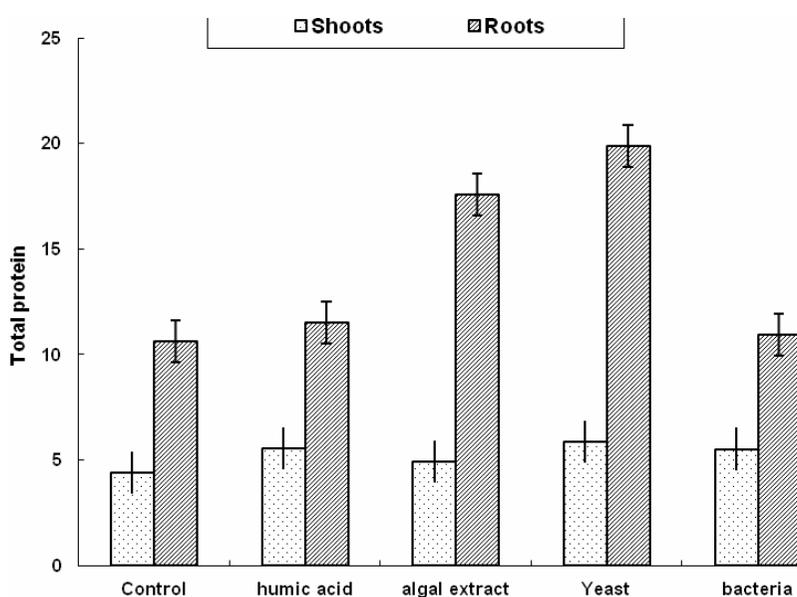


Figure 4. Effects of bacteria, yeast, algal extract and humic acid on total protein contents of field *Vicia faba* CV. Giza 3 beans

Nutrient concentrations:

All treatments showed partially significant differences among nutrient concentrations uptake in shoots and roots of field bean (Table 3). All studied treatments significantly increased magnesium, nitrogen and phosphorus concentration in field bean plants sample as compared with control plants. However, the highest nitrogen and phosphorus content were recorded with bacteria strain as compared with the control. On the other hand, the algae extraction treatment recorded the highest values of phosphorus. Moreover, calcium concentration decreased by the algae extraction treatment compared with untreated and treated in shoot plants. The soil applications of biostimulants have significant effects on the uptake of nitrogen in field bean as compared with the control.

The addition of humic acid improved N and P concentrations in wheat and maize plants by lowering the soil pH value through yielding organic acid as well as increasing the activity of soil organisms and preventing nutrient ions from leaching [16]. Seaweed liquid

fertilizers improved nutrient uptake, and are considered a good source of nutrients for the plants this liquid provide nutrients to the plant and enhance the yield of plants [23].

Yeast extract increased NPK, content in seeds of field beans and increase in nitrogen, potassium, phosphorus and calcium contents in tomato plants [23]. These organisms also facilitate the growth of plants by improving the uptake of nutrients and production of some phytohormones and convert insoluble form of phosphorous into soluble one enhancing phosphorous availability to plants. [12].The objective of this research was to provide the potential advantages of biostimulants as organic matter on growth and development of plants.

Table 3. Effects of humic acid ,algal extract, yeast and bacteria on nutrient concentrations of field *Vicia faba* CV. Giza 3 beans

Treatments	Potassium mg%		Nitrogen mg%		Phosphorus mg%		Calcium mg%		Magnesium mg%	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Control	1.367 ±0.03	0.653 ±0.04	0.883 ±0.08	1.751 ±0.07	0.220 ±0.02	0.173 ±0.07	0.882 ±0.08	0.348 ±0.06	66.440 ±1.45	75.190 ±1.67
Humic acid	1.488 ** ±0.04	0.790 ** ±0.05	1.094 ** ±0.09	1.700 ** ±0.08	0.250 ** ±0.05	0.195 ** ±0.04	1.015 ** ±0.09	0.420 ** ±0.04	67.990 ** ±1.67	80.740 ** ±1.46
Algal extract	1.745 ** ±0.02	1.352 ** ±0.07	1.241 ** ±0.05	2.898 ** ±0.09	0.296 ** ±0.04	0.290 ** ±0.03	0.827 ** ±0.07	0.606 ** ±0.02	79.380 ** ±1.56	76.030 ** ±1.24
Yeast	1.402 ** ±0.05	1.198 ** ±0.06	1.161 ** ±0.04	2.810 ** ±0.03	0.389 ** ±0.06	0.186 ** ±0.02	1.928 ** ±0.06	0.529 ** ±0.05	73.710 ** ±1.31	78.230 ** ±1.32
<i>Bacillus licheniformis</i>	1.508 ** ±0.06	0.873 ** ±0.07	2.898 ** ±0.03	2.944 ** ±0.04	0.730 ** ±0.05	0.394 ** ±0.05	1.873 ** ±0.03	0.478 ** ±0.03	67.990 ** ±1.25	80.740 ** ±1.55
L.S.D. %5at	0.02	0.04	0.12	0.09	0.03	0.01	0.08	0.01	0.77	0.37

** Highly significant change

Conclusion

From the above mentioned results it can be concluded that, the application of the biostimulants in small quantities has been effected on several metabolic processes, enhances plant growth and development via the increasing of photosynthesis, endogenous hormones, ion uptake, and protein synthesis as well as with relatively higher ability for increasing available micronutrients in the soil. The beneficial effect and advantages of such products (Bacillus, Yeast, algal extract and humic acids) as organic matter due to their uses for production organic farming , decreased the using and dependence on chemical fertilizers as well as on growth and development of field bean plants .

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