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Can foliar application of natural biostimulants reduce nitrate and fiber content in fresh green bean under soil nutrient deficiency?

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Abstract

Background There is a growing body of literature that recognizes the importance of natural biostimulants in the agricultural productivity. This potential can play an important role in addressing the issue of cultivation on sandy soil, which is well-known with soil nutrient deficiency stress. In the current study, the selected biostimulants (i.e., chitosan, amino acids, yeast extract, and humic acid) were foliar applied on green bean in the open field of alkaline sandy soils.

Results The main results that obtained after harvesting the fresh green bean pods were confirmed that all studied biostimulants improved the quantity and quality of green bean production compared to the control. Chitosan was the best applied biostimulants for producing green bean in a lower fiber and nitrate content of pods, a higher crude protein content of pods, and higher NPK content in the leaves.

Conclusion The role of studies biostimulants in promoting green bean productivity under nutrient deficiency stress may back to increase the uptake of nutrients (NPK), plant physiological (higher dry matter) and biochemical attributes (higher crude protein). More studies are needed using different applied doses and more different kinds of natural biostimulants.

Keywords Chitosan, Amino acid, Yeast, Humic acid, Nitrogen deficiency, Fiber content, Nitrate content

Background

The term “plant biostimulants” can be defined as any material or microbe can be applied to plants for enhancing the efficiency of plant nutrition, its tolerance to abiotic stress and the quality of crop traits, without regarding to their content of nutrients (du Jardin 2015). These biostimulants do not include the category of pesticides, fertilizers, or biocontrol agents, but they have the ability to improve the nutrition of cultivated plants (Neshevet et al. 2022; Muhie 2022). Plant biostimulants may classify into categories such as humic substances, seaweed extracts, chitosan, protein hydrolysates and amino acids, biopolymers, beneficial microbes (bacteria, and fungi), and beneficial elements (i.e., aluminum,

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cobalt, sodium, selenium and silicon) (du Jardin 2015; Yakhin et al. 2017; Rouphael and Colla 2020). Application of chitosan had notably been alleviated plant oxidative stress to promote crop productivity under stress (Hidangmayum et al. 2020; Balusamy et al. 2022; Hidangmayum and Dwivedi 2022; Ji et al. 2022). Chitosan has the ability to improve physiological attributes of stressful plants, higher nutrient uptake, cell division and synthesis of proteins (Hidangmayum and Dwivedi 2022). Humic substances have many benefits for agro-ecosystem (Tiawari et al. 2022), which could be recovered from the sewage sludge (Michalska et al. 2022) or as microalgal biostimulants (Popa et al. 2022). Amino acids and their derivatives also can mediate plant defense under stress (Cai and Aharoni 2022). Enormous reports confirmed the role of beneficial elements (mainly Se and Si) under stressful conditions (Gui et al. 2022; Kapoor et al. 2022).

The common or green beans or snap beans are vegetables can consume as fresh green pods or as seeds (García-Fernández et al. 2022). Green bean (*Phaseolus vulgaris* L.) as a legume crop, is the highest direct consumption in the world by more than 300 million people (Palacio-Márquez et al. 2021). This crop is considered a main source for plant protein, and high content of minerals (mainly calcium, iron, potassium, manganese, magnesium, phosphorus, and zinc) (García-Fernández et al. 2022). Common beans in form of green pods are important for human health, because of its antioxidant activity, which have anti-inflammatory, anti-diabetic, anti-mutagenic, anti-obesity, and anti-carcinogenic attributes (Palacio-Márquez et al. 2021; García-Fernández et al. 2022). The cultivation of common bean under stress leads to decrease the yield, which can promote by applying some biostimulants including the organic and inorganic sources such as chitosan (Palacio-Márquez et al. 2021), and selenium (Mansoor et al. 2023). Many studies carried out on the cultivation of green bean under stress such as salinity (Azizi et al. 2022), drought stress (Ziaei and Pazoki 2022), phosphorus stress (Mansoor et al. 2023), and cold stress (Yang et al. 2023). A few studies on common bean production and nutrient deficiency stress have issued like phosphorus stress (Smith et al. 2022). In contrast to this, there is several studies on this crop productivity under toxic nutrients could be found such as arsenic (Shah et al. 2022), cadmium (Hediji et al. 2021; Koleva et al. 2022), and heavy metals (Hammami et al. 2022).

Therefore, this study makes a major contribution to research on natural biostimulants by demonstrating their impacts on green bean productivity under sandy soil conditions. Which can be used under such studied conditions. Foliar application of chitosan, amino acid, yeast,

and humic acid were investigated on yield and quality of green bean under nutrient deficiency stress.

Methods

Experimental site and treatments

A field experiment was carried out at EL-Nubaria, El-Behira Governorate, Northern Egypt, at the experimental station of National Research Centre, during two successive summer seasons of 2021 and 2022. Foliar spray of some natural bio-stimulants was tested on growth and yield of green bean "Paulesta cv." On the last week of February on 2021 and 2022, the seeds were sown, and harvested on the last week of April in both seasons. Seeds were also sown on two sides of ridge, where each ridge was 80 cm width and 4 m length and 10 cm apart. Each plot included 4 ridges and the plot area was 12.8 m². Figure 1 presents the main treatments including the control, chitosan and amino acids and humic acid at 2 ml L⁻¹, whereas, the yeast extract at 2 g L⁻¹. All foliar application treatments were sprayed twice after 30 and 40 days from sowing. The soil has sandy soil texture (silt 0.66, clay 9.26 and sand 90.08%), EC was 1.80 dS m⁻¹, and soil pH was 8.25. The main characterization of used biostimulants in the current study is presented in Table 1. The agricultural practices were commonly followed in the farm for as the recommended for bean production.

Vegetative parameters and chemical analyses

A random sample of five plants from each plot was taken after 50 days from sowing to record the following vegetative growth characters as shown in Fig. 1. After 60 days from sowing and at harvest stage, the mature pods of bean for each experimental plot were collected and recorded as ton fed⁻¹. The average pod fresh weight and pod length were recorded using 50 pods from each plot. Samples of leaves were oven dried at 65 °C then fine grinded and wet digested to measure N, P and K. According to Page et al. (1982), the N, K, P were determined using Kjeldahl method, flame photometer (NADE LCD Digital Flame photometer FP640, China), and the visible spectrophotometer (Single Beam, SPIV722N, 721N, China), respectively.

Quality, yield of green bean and its components

The quality of cultivated green bean was evaluated by measuring the fiber content (%) in pods was determined according to Rai and Mudgal (1988), nitrate and protein content as well as the length and fresh weight of harvested pods. Nitrate content in pods was measured according to Cataldo et al. (1975) and modified by Li et al. (2021). After measuring the N, the crude protein was calculated by multiplying by the 6.25 (N×6.25) conversion factor, and

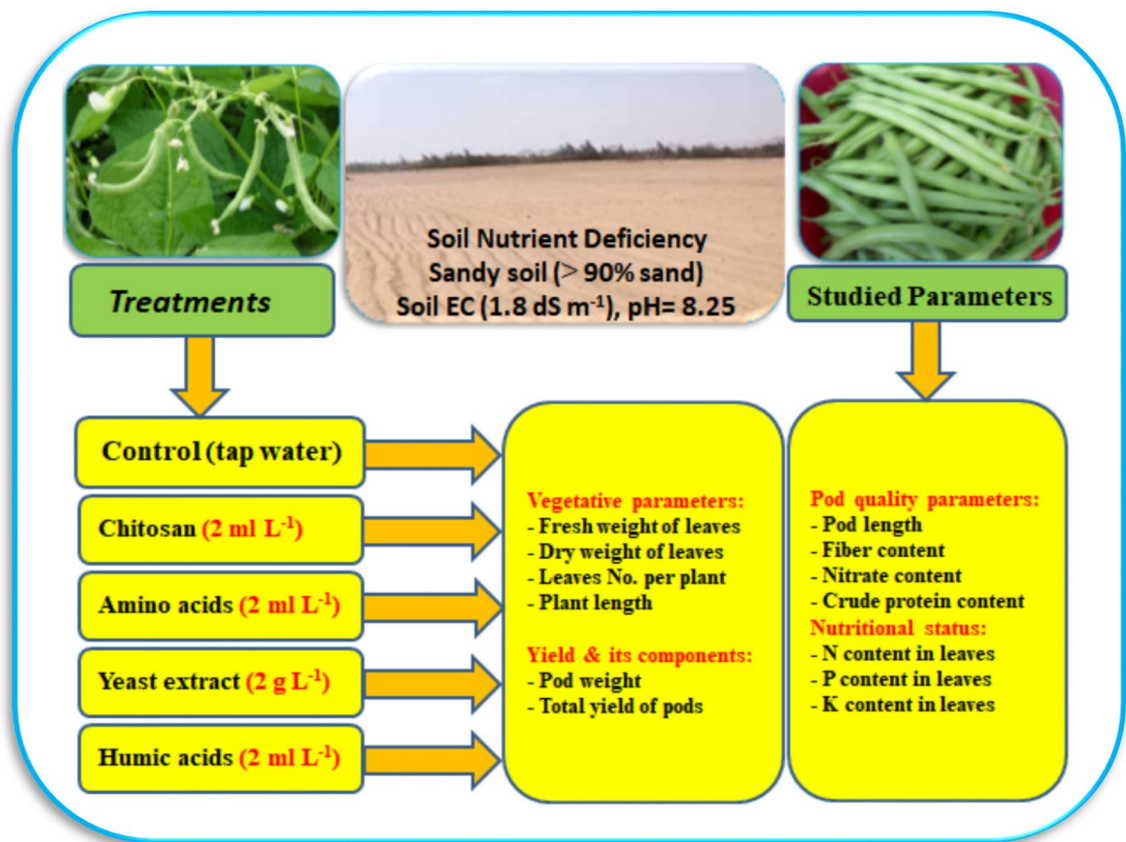


Fig. 1 An overview on the main applied treatments in the study including different applied doses and the studied parameters

Table 1 The main characterization of used biostimulants in the current study

Used biostimulants	The source	Main bioactive compounds
Chitosan	Local commercial product of "Chito-Care"	Plant growth promoter
Amino acids	Commercial product "Amino total"	17 different amino acids (%): mainly of glutamic (7.2–9.1), serine (3.7–4.4), arginine (5.2–6.2), etc
Yeast extract	Commercial product of pure dry yeast powder	A mixture of amino acids, peptides, and water-soluble vitamins (like B12)
Humic acids	Commercial product of humic acid 25 g L ⁻¹	Folic acid (0.7 g L ⁻¹), nitrogen (4.0 g L ⁻¹), phosphorus (0.6 g L ⁻¹) and potassium (8.0 g L ⁻¹)

the results were then calculated as a percent according to AOAC (1990).

Statistical analyses

At the confidence level of 5%, all data of the experiment were statistically analyzed on means of treatments to measure the considered significantly different according to Gomez and Gomez (1984).

Results

Vegetative parameters of green bean

Before harvesting, some selected vegetative parameters were measured including leaves dry and fresh weight, and No. of leaves /plant (Table 2). Plant length, pod length and its weight are presented in Table 3. From the data in Tables 2 and 3, there were differences between the studied vegetative parameters and different applied

Table 2 Effect of biostimulants on vegetative growth parameters

Treatments	Leaf dry weight (g)		Leaf fresh weight (g)		No. of leaves /plant		Plant length (cm)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	7.34 e	10.57 d	93.17 e	100.33 b	34.35 c	37.25 d	44.23 c	47.17 c
Chitosan	12.86a	16.54 a	125.56 a	145.94 a	39.27a	43.64 a	48.36 a	53.79 a
Amino acids	11.65b	14.48 b	109.46b	128.75 ab	37.48ab	41.87 b	46.33 b	49.36b
Yeast extract	10.37c	13.16 c	102.35d	122.93 ab	36.50bc	38.68c	46.12 b	48.78bc
Humic acids	9.68 d	12.27 c	104.67 c	117.68 ab	37.70ab	37.58 cd	45.25bc	47.18 c
F-test	**	**	**	**	**	**	**	**

** Indicates highly significant treatment and values of means in each column followed by the same letter are not significantly at level of $p < 0.01$

Table 3 Effect of biostimulants on yield of green bean

Treatments	Pod length (cm)		Pod weight (g)	
	2021	2022	2021	2022
Control	11.60 d	12.25	4.60	4.70 b
Chitosan	13.06 a	13.64	5.76	5.94 a
Amino acids	12.26 c	12.87	5.54	5.75 a
Yeast extract	12.70 b	12.68	5.68	5.93 a
Humic acids	12.70 b	12.58	5.67	5.68 a
F-test	**	NS	NS	**

** Indicates highly significant treatment and values of means in each column followed by the same letter are not significantly at level of $p < 0.01$, whereas NS indicates not significant

biostimulants. The highest values were obtained after foliar chitosan application followed by amino acid. The more surprising correlation is with the applied chitosan, which recorded the highest values in all studied

vegetative parameters (12.86 and 16.54 g), (125.56 and 145.94 g), (39.27 and 43.64), (48.36 and 53.79 cm), (13.06 and 13.64) and (5.76 and 5.94) for leaves dry and fresh weight, No. of leaves /plant, plant length, pod length and its weight in both seasons, respectively.

Yield of green bean, pod quality and its nutritional status

The yield of green bean and its quality are tabulated in Fig. 2. The studied yield parameters included pod length, and weight, beside the total yield of green bean. All studied biostimulants significantly increased the yield of green bean compared to the control with priority to chitosan. Chitosan gave the highest values with a significant increase in pod length (13.06 cm) and beside the total yield of green bean (4.79 ton fed⁻¹), whereas this increase was not significant for the pod weight (5.94 g). The quality of green bean pods was evaluated by measuring the fiber and nitrate content, beside the crude protein content (Table 4). Applied biostimulants were increased

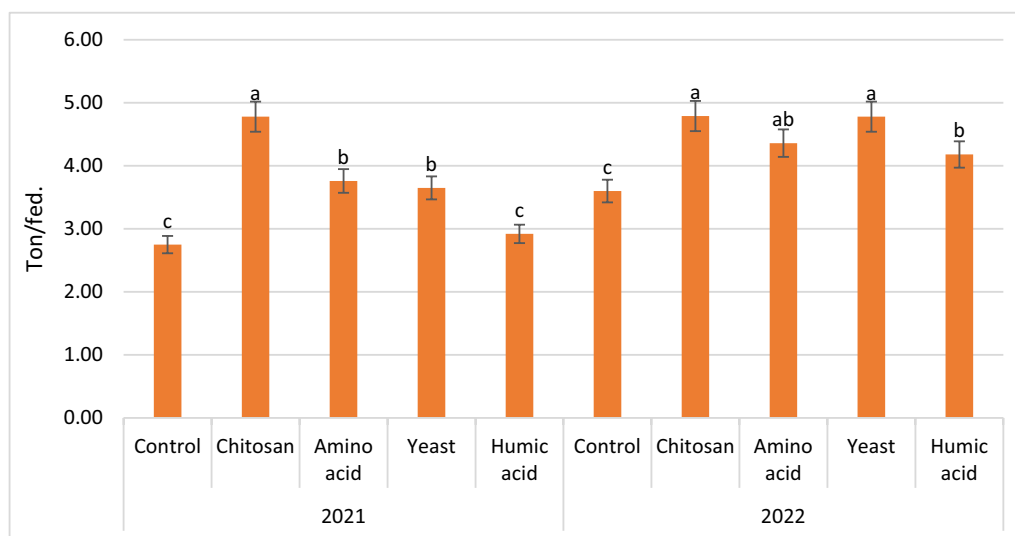


Fig. 2 Effect of foliar spray of natural stimulated compounds on yield (ton/fed) of green bean plant in 2021 and 2022. Each column followed by the same letter are not significantly at level of $p < 0.01$

Table 4 Effect of foliar spray of biostimulants on yield quality of fresh green bean pods in both seasons

Treatments	Crude protein content (%)		Nitrate content (%)		Fiber content (%)	
	2021	2022	2021	2022	2021	2022
Control	14.31 c	10.19 c	0.74 a	0.80 a	13.35 a	12.57
Chitosan	18.00 a	12.38 a	0.52 b	0.56 c	11.86 c	11.54
Amino acid	15.75 b	10.68 bc	0.68 a	0.72 ab	12.65 b	12.48
Yeast extract	17.93 a	11.56 ab	0.57 b	0.60 bc	11.37 d	12.16
Humic acid	15.88 b	11.13 ab	0.52 b	0.66 b	12.68 b	12.27
F-test	**	**	*	**	**	NS

*, and ** indicates highly significant treatment and values of means in each column followed by the same letter are not significantly at level of $p < 0.05$ and $p < 0.01$, whereas NS indicates not significant

the crude protein in pods comparing with the control, whereas the opposite was found in case of fiber and nitrate content in bean pods (Table 4).

The highest values in crude protein were noticed after foliar applying chitosan to be 18.00 and 12.38%, in both seasons, respectively. On the other hand, chitosan also recorded the lower values in both nitrate and fiber of pods in both seasons compared to the other applied biostimulants and control. It is worth to notice that all applied biostimulants were recorded significant differences among them concerning both crude protein and nitrate, whereas non-significant for fiber content in 2022 season only. The nutritional status of NPK in green bean leaves is reported in Table 5. The foliar application of chitosan led to increase the nutrient content (NPK) in green bean leaves with high significant differences in season of 2021 for all studied nutrients. It could be concluded our results in the following Fig. 3, which explain more clarification on the suggested mechanism of our results.

Discussion

The production of green bean under stress is considered a real challenge facing the cultivation of this very important crop, which lead to decrease the productivity. The current study represents production of green bean under sandy soils (more than 90% sand), which suffers from the

stress of nutrient deficiency. Some selected biostimulants were investigated in ameliorating this stress including chitosan, yeast extract, amino acids, and humic acids. In two successive seasons (2021 and 2022), a field experiment was carried out under sandy alkaline soil. In this section, it would be nice to answer the main questions, which one likes to ask on it: what is the main role of applied biostimulants to promote the vegetative growth and production of green bean under soil nutrient deficiency? Which biostimulant was the best in enhancing the productivity of green bean under such stress? Can applied biostimulants reduce the content of green bean pods from nitrate and fiber?

It is well known that any stress can cause a problem in the growth and productivity of the green bean like water deficit (Galvão et al. 2019). Many biostimulants have the ability to support green bean production under such stress through producing phytohormones, which may improve root growth induction, the contribution of nutrients, and regulate antioxidant systems (Galvão et al. 2019). In the current study all studied biostimulants improved the growth and productivity of green bean compared to the control, where the priority was noticed for the chitosan. Chitosan has a distinguished non-toxic, biodegradable, and biocompatible compound that promotes physiological and biochemical attributes

Table 5 Nutritional status in dried bean leaves after foliar spray biostimulants in both seasons

Treatments	N content (%)		P content (%)		K content (%)	
	2021	2022	2021	2022	2021	2022
Control	2.29 c	1.63 c	0.72 c	0.70	1.03 d	0.89
Chitosan	2.88 a	1.98 a	0.95 a	0.83	1.70 a	1.31
Amino acid	2.52 b	1.71 bc	0.93 a	0.74	1.32 c	0.94
Yeast extract	2.87 a	1.84 ab	0.94 a	0.87	1.53 b	1.09
Humic acid	2.54 b	1.78 ab	0.88 b	0.77	1.51 b	0.92
F-test	**	**	*	NS	**	NS

* and ** indicate significant and highly significant treatment and values of means in each column followed by the same letter are not significantly at level of $p < 0.05$ and $p < 0.01$, whereas NS indicates not significant

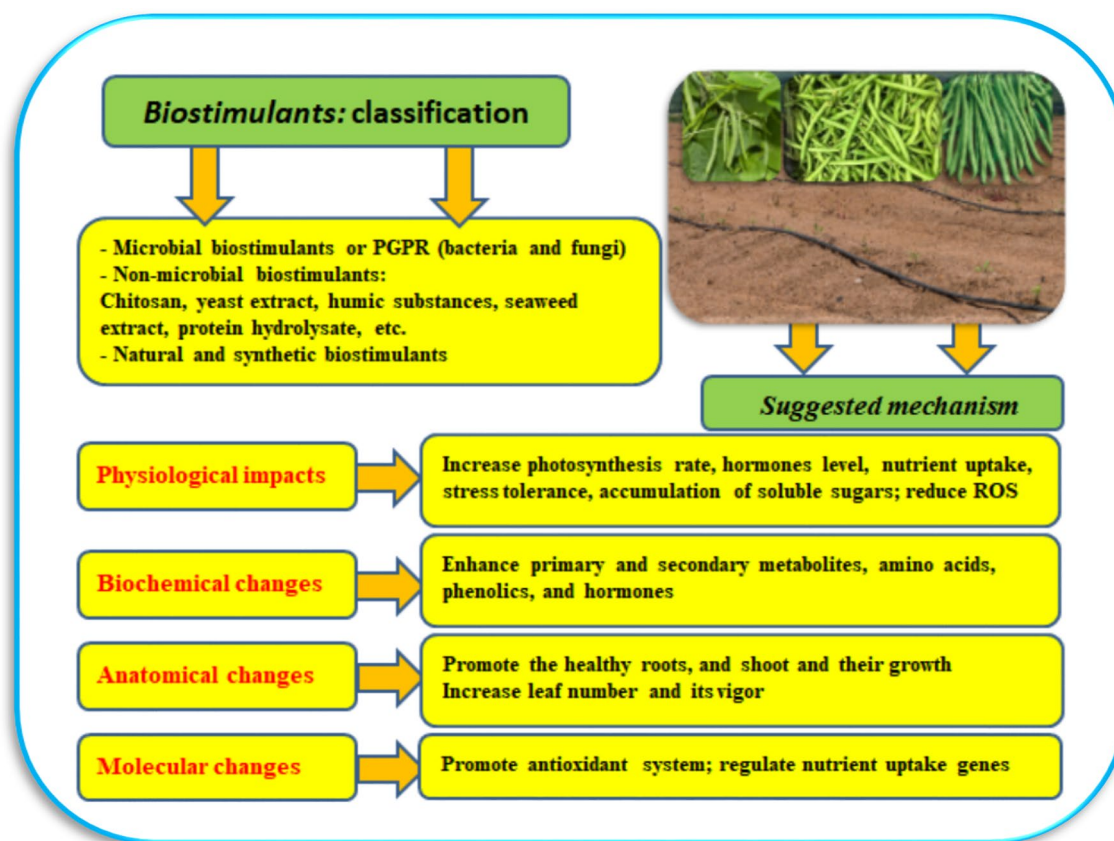


Fig. 3 General overview on the biostimulants, their classification, and the suggested mechanisms

of the stressful plants (Balusamy et al. 2022). The increasing yield of green bean was also confirmed by Palacio-Márquez et al. (2021), who reported that using chitosan has positive effects on photosynthetic pigments, vegetative growth, and the yield under stress.

Which biostimulant was the best in enhancing the productivity of green bean under such stress? According to the current study, chitosan was the best biostimulant in promoting the productivity of green bean under soil nutrient deficiency. The reason may be due to the used concentration, which was effective besides the several advantages of chitosan under such stress to support cultivated bean plants. Chitosan is considered an excellent plant growth promoter and green pesticide due to its non-pollution, biodegradation, and non-toxic characteristics (Ramzan and Younis 2022). More reasons may include the ability of chitosan to alleviate plant oxidative stress to promote green bean yield, and the mode of action of chitosan on stressful plants and its defense system mainly depends on the method of application and applied dose (Ji et al. 2022). The obtained results were in agreement with results of Agüero-Esparza et al. (2022). The used biostimulants have the ability to support stressful plants through their

role as bioactive components and phytohormones (Rady et al. 2019). Many studies reported about the crucial role of biostimulants in enhancing stressful plants under different stress such as drought (Ramzan and Younis 2022), water deficit (Galvão et al. 2019; Hernández-Figueroa et al. 2022), salinity (Rady et al. 2019), etc.

Can applied biostimulants reduce the content of fresh green bean pods from nitrate and fiber? It is well-known that the accumulation of nitrate in the edible vegetables or fruits is a serious global issue, which threatens human health (Haftbaradaran et al. 2018). The main factors that control the accumulation of nitrate in vegetable tissue may include the soil characters, water, and organic fertilizers (Ortega-Blu et al. 2020). So, an increase in concern on the accumulation of nitrate in vegetables due to excessive chemical fertilizers and unreasonable farming practices. The converting nitrate after intake by human into nitrite causing diseases of gastric carcinoma or methemoglobinemia (Luo et al. 2022). The applied biostimulants reduced the accumulation of nitrate in green bean pods compared to control with higher reducing rate in nitrate for chitosan. It is found that applied biostimulants did not modulate nitrate accumulation in lettuce

(Ottaiano et al. 2021). Concerning the fiber content, it is preferable to decrease this content when the pods will be consumed freshly. In our study, all studied biostimulants decreased the fiber content in bean pods with higher rate to chitosan. This result is a harmony with results of El Sheikha et al. (2022), who confirmed that applied biostimulants (i.e., yeast extract, humic acid and moringa) improved the nutritional value including protein and carbohydrates. This result also could be achieved using the synthetic biostimulants (Szparaga et al. 2019). Concerning the content of dietary fiber in fresh bean seeds, still needs more explanations because the effect of biostimulants on plants is not only a consequence of direct regulation of metabolism, but also of their multifaceted actions (Bhupenchandra et al. 2022). This role of biostimulants in mitigating the effects of stressful conditions on crop productivity for sustainable agriculture (Kaushal et al. 2023).

It could be concluded that natural biostimulants can offer novel possibilities in agriculture through improving crop productivity, although their mechanism still needs more studies (Szparaga et al. 2019). Different approaches can be handled to focus on the mode of action of biostimulants including the physiological, anatomical, biochemical and molecular tools. Plant antioxidants system and hormones that resulted from applying biostimulants may improve metabolic processes in plants without modifying their natural pathways (Posmyk and Szafranska 2016). The role of biostimulants under reduced nutrient supply by enhancing quality of ornamental plants was confirmed by Loconsole et al. (2023). The window still opens for more studies on the natural and synthetic biostimulants especially under different stresses as reported by several recent studies (e.g., Rai et al. 2021; Bhupenchandra et al. 2022; Loconsole et al. 2023).

Conclusions

The global production of green beans needs to be increased to meet the over-increase in the global population due to its high consumption by humans. Abiotic stress like soil nutrient deficiency stress (mainly in the sandy soils) is a serious obstacle for green bean production under such stress. The current study carried out to evaluate different applied natural biostimulants including chitosan, amino acids, humic acids, and yeast extract under nutrient deficiency stress. All studied biostimulants improved the growth and production of green bean under such stress compared to the control. The distinguished results were recorded by chitosan more than other biostimulants, which was recorded the highest values of studied parameters especially the yield and its components. Reducing the content of fiber and nitrate in fresh green beans was one of the main aims which also

was achieved after applying all studied biostimulants with priority to chitosan. More studies are needed for more information about the bioactives in applied biostimulants, which can cause such synergistic effect.

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Author contributions

This work is a combined effort of all of the authors. ZF and H M designed this work, conducted the field experiments, and performed the chemical analysis of the samples. M A and H R wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are included in this published study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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