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RESEARCH ARTICLE

# Effect of biostimulants on soil characteristics, plant growth and yield of Pea (*Pisum sativum* L.) under field conditions

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## Abstract

Because of the safe use of biostimulants as they are eco-friendly and modern technique for substituting chemical fertilizers which have negative impacts on the environment. Therefore, this study was conducted to determine the effect of two biostimulants, *Spirulina platensis* and *Trifolium alexandrinum* on soil properties, growth and yield of pea (*Pisum sativum* L.) grown under silty clay soil conditions. The field experiment was carried out during the winter season by applying each fertilizer to the cultivated soil at 3 concentrations (2.5, 5 and 10 g) either singly or in combination, forming 15 treatments in addition to control. It was found that the vegetative growth parameters as well as, yield components were significantly increased by two fertilizers applied singly or in combination. In comparison with control, the highest concentration of combined treatment (10 g) significantly increased shoot length, number of leaves, leaf area and number of branches by 145, 200, 300 and 100 % respectively. Furthermore, the combined treatment (10 g) showed the highest values of the number of pods (8), pod length (12 cm), no. of seeds (13) and dry weight of seeds (21.5 g), compared to control. Biostimulants boosted the plant growth and yield by enhancing the estimated parameters. Also, the physicochemical properties and mineral status of soil were improved after the application of the biostimulants. The current study indicates that the single and combined treatments of *Spirulina platensis* and *Trifolium alexandrinum* may potentially increase soil fertility and enhance the growth and yield of pea.

## Keywords

*Trifolium alexandrinum*, *Spirulina platensis*, soil fertility, mineral status, yield components

## Introduction

The overuse of chemical fertilizers during the previous 150 years has left soils deteriorated, polluted and less productive as well as posing serious health and environmental risks. Organic farming practices which use biostimulants in agriculture, would solve these problems and improve the ecosystem's health. Living microorganisms or natural substances generated from bacteria, fungi and algae are used as biostimulants to improve soil chemical and biological parameters, promote plant development and restore soil fertility (1). They are particularly important in improving soil fertility since they fix atmospheric nitrogen both with and without plant roots, solubilize insoluble soil phosphates, and secrete plant growth chemicals in the soil (2). In terms of sustainable agriculture, Cyanobacteria (*Spirulina* *platensis*) can help by boosting soil fertility, crop growth and yield, and improving environmental quality (3). It has been proven that cyanobacteria have the ability to fix atmospheric nitrogen into usable forms for plants and hence, affect rice fields (4). Moreover**,** They produce bioactive substances such as (polysaccharides, growth hormones and antimicrobial compounds) that promote plant development, making them viable alternatives for biostimulants (5). Also, several reports showed some examples of plant biostimulants that benefit the growth and yield of many crops such as moringa leaves extract (6), garlic cloves (7), *Lolium perenne* (8) and oak (9). It is well known that legume application as a biostimulant not only increases total soil organic C and N content but also reduces negative environmental impacts (10). Soil incorporated with plant residues from green manure showed activation of the physicochemical protection of organic carbon (11) and this triggers plant health by improving plant nutrition. *Trifolium alexandrinum* (*Trifolium* clover) is a highly nutritious forage due to its high amounts of nutrients, particularly protein (15-25% DM), minerals (11-19%) and carotene (12). Additionally, it is rich in trace elements such as Fe, Zn, Mn and Se. Regarding micronutrients application, it was found that applying small levels of Zn, Fe and Mn via foliar spraying enhanced crop yield considerably (13). Zn is recognized to play a significant part in a wide range of enzymes, either as a metal component or as a cofactor (14). Where Fe plays an essential role in photosynthesis (15). Although Se is not regarded as essential for plants, its use in crops is usually effective for growth and stress tolerance (16). Also, according to the reports (17), microelement fertilization enhances seed yield due to causing an increase in the number of pods/plant and with a smaller contribution from an increase in the number of seeds per pod.

The high nutritional value makes legumes, especially pea (*Pisum sativum* L.) is one of Egypt's most important vegetable crops for local use and export during the winter season. Pea grains could play a key role in organic cropping systems in addition to the pods rich in protein and carbohydrates. As a result, pea is regarded as one of the most significant food sources for humans (18).

The present study aimed to highlight the prospects of *Spirulina platensis* and *Trifolium alexandrinum* as options for biofertilization and as agents for improving soil structure and functioning and enhancing growth performance and yield of pea.

## Materials and Methods

### Seeds and study area

Healthy and viable seeds free from visible infection with uniform size of pea (*Pisum sativum* L.) cv. Master B were obtained from Horticultural Research Institute Center of Egypt. Before sowing, seeds were surface sterilized with acidified 0.01% $HgCl_2$ for 5 min. and after serial washings with sterilized water.

This experiment was carried out under field conditions in an agricultural land lies at 30° 36' N Latitude and 31° 28' E Longitude, west Zagazig University, Sharqia governorate, Egypt during winter (October /March). The soil used in this study had the following properties: mechanical analysis (sand 4.6, silt 35 and clay 59.4 %), chemical analysis with (pH 8.2, EC 3.4 $dSm^{-1}$ and organic matter 1.2 %), soluble cations (Ca 36.2, Mg 6.2, Na 0.88 and K 0.8 mmolc $L^{-1}$) and available nutrients were N 246.3, P 4.99 and K 457.2 mg $kg^{-1.}$

Table 1. **Table 1.** Nutrient analysis of *Trifolium alexandrinum*

| Crude Protein % | soluble Carbohydrates % | Available N % | Micro element (mg 100 $g^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Fe | Zn | Cu | Mn | Se |
| 28.4 | 2.64 | 0.051 | 1.57 | 1.15 | 1.09 | 3.02 | 0.42 |

### Experimental design

Before cultivation, the field was plowed and laid out into four rows. Each row has 16 lines (each was 3.5 m×80 cm). Each soil line was applied with 2 fertilizers, *Spirulina platensis* (1st) and *Trifolium alexandrinum* (2nd) separately and in combinations of the 2 fertilizers. Three concentrations (2.5, 5 and 10 g) of each fertilizer were applied during soil preparation in a split-plot design with three replicates, This experiment included 16 treatments which included all combinations between the three levels of 2 fertilizers in addition to control. Seeds were sown in 10th October and the normal agriculture practices of pea under drip irrigation system were followed according to the recommendations of the Agriculture Ministry. During the experimental period which lasted for 4 months, the studied parameters were recorded weekly.

### Chemical analysis of biostimulants

Quantitative estimation of active principles in powder of whole plant, *Trifolium alexandrinum* showed in (Table 1). *Spirulina platensis* is rich in protein (58.2 %), lipids (23.2 %), carbohydrates (24.5 %), vitamins and minerals (2.7-3 %) (19).

### Growth characteristics and yield

Growth parameters like shoot length (cm), leaf area ($cm^2$), number of branches and the number of leaves per plant were recorded in 3 plants selected from each line. Samples were taken randomly after 45 days from sowing.

At the harvest period, pea samples were taken to determine the yield components like the number of pods $plant^{-1}$, pod length (cm), number of seeds $pod^{-1}$ and weight of 100 seeds (g).

### Soil analysis

Soil samples were collected at two depths (0-15 and 15-30 cm) from the highest concentration of both fertilizers (10 g) in addition to control. This treatment was chosen due to it was the most effective concentration on plant growth and yield. These samples were carried to laboratory in plastic bags and spread over paper sheets, air dried then passed through 2 mm sieve to remove gravel and debris then packed in plastic bags ready for physical and chemical analysis according to (20).

Macro and micro nutrient contents were determined in soil samples according to standard procedure (21). The elements were measured by flame photometer Shimadzu Model AA 640 F (Japan). While, cations (calcium, magnesium, potassium and sodium) were determined of soil extraction (1:5) by standard procedures (22).

### *Statistical analysis*

Statistical analysis was carried out according to standard procedure (23), the p-value < 0.05 was considered statistically significant. The differences between the mean values of various treatments were compared by Duncan's multiple range test (DMRT).

## Results

### *Vegetative growth characteristics*

All the studied growth parameters were significantly increased by increasing the concentrations of 1st fertilizer, *Spirulina sp* compared to control. The same trend occurred under the application of the 2nd fertilizer (*Trifolium*). Further increases occurred after the combination of the two fertilizers (Table 2). The maximum values of the estimated growth parameters were recorded at the combined treatment of the highest concentration (10 g). The maximum values of shoot length, number of leaves, leaf area and number of branches were 40.5 cm, 13.5, 23.5 $cm^2$ and 3 respectively.

**Table 2.** Growth parameters under effect of different concentrations of *Trifolium alexandrinum* and *Spirulina platensis*

| Fertilizers (g) | | Parameters | | | |
|---|---|---|---|---|---|
| *T. alexandrinum* | *S. platensis* | Shoot length (cm) | No. of leaves $plant^{-1}$ | Leaf area ($cm^2$) | No. of branches $plant^{-1}$ |
| 0 | 2.5 | 29.95±0.05** | 7.5 ±0.5** | 13.6± 0.6** | 2.34 ± 0.47 ns |
| 0 | 5 | 30.5±0.5** | 8.5±0.5** | 15.25±0.25** | 3.5±0.5** |
| 0 | 10 | 31.75±0.25** | 9.5 ±0.5** | 16.4±0.4*** | 2.66±0.00* |
| 2.5 | 0 | 32.5 ±0.5** | 9.5 ±0.5** | 16.65±0.15*** | 2.5±0.5 ns |
| 5 | 0 | 33.75±0.25*** | 10 ± 0.00*** | 17.15±0.15*** | 3.5±0.50 |
| 10 | 0 | 35.5 ±0.5*** | 10.5±0.5*** | 17.55±0.05*** | 3±0.81 ns |
| 2.5 | 2.5 | 35.1 ± 0.4*** | 9.5 ±0.5** | 18.25±0.25** | 2±1.00 ns |
| 5 | 2.5 | 35.25±0.25*** | 10.5±0.5*** | 18.6 ±0.1*** | 2.5±0.5 ns |
| 10 | 2.5 | 36.75±0.25*** | 11.5±0.5** | 19.15±0.15*** | 2.5±0.5 ns |
| 2.5 | 5 | 36.5 ± 0.00*** | 11± 0.00*** | 19.7±0.21*** | 2.5±0.5ns |
| 5 | 5 | 36.75±0.25*** | 11.5±0.5** | 20.85±0.15*** | 3 ± 0.00** |
| 10 | 5 | 37.75±0.25*** | 12.5±0.5*** | 21.75±0.25*** | 3 ±0.00** |
| 2.5 | 10 | 37.75±0.5*** | 11.5±0.5** | 22.25±0.25*** | 3±0.00** |
| 5 | 10 | 39.5±0.5*** | 12.5±0.5*** | 22.5±0.5*** | 3±0.00** |
| 10 | 10 | 40.5±0.5*** | 13.5±0.5*** | 23.5±0.5*** | 3±0.00** |
| Control | | 16.5±1.5 | 4.5±0.5 | 5.85±0.85 | 1.5 ±0.5 |

Mean± standard errors based on ANOVA analysis, *significant, P<0.05; **significant, P <0.01; ***significant, P<0.001 and Ns is nonsignificant difference.

### *Effect of biostimulants on yield*

Data in (Table 3) clearly indicated that the yield components like number of pods $plant^{-1}$, pod length, number of seeds $pod^{-1}$ and dry weight of 100 seeds were estimated. The results showed significant increases in all the estimated yield components treated with either single or combined biostimulants. Generally, the highest concentration of combined biostimulants gave the highest values of yield, Number of pods $plant^{-1}$ (8), pod length (12 cm), no. of seeds $pod^{-1}$ (13.5) and the dry wt. of 100 g seeds were (21.2 g).

### *Physicochemical properties of soil*

Regarding the physical and chemical analysis of soil (Table 4) clarified that the physical properties include particle size, bulk density (BD), coefficient of linear extensibility (COLE), hydraulic conductivity (HC) and Water dispersible clay (WDC). The particle size distribution in soil clarified that the type of soil in the two selected treatments was silty clay. Regarding bulk density and COLE in the two treatments either at surface soil (0-15 cm) or at subsurface soil (15-30 cm) not greatly differ but still more than its values in control since BD at depth (0-15 cm) was 1.43, 1.4 $mg/m^3$ and at depth (15-30 cm) was 1.4, 1.3 $mg/m^3$. Whereas, COLE at depth (0-15 cm) was 1.34, 1.36 while at depth (15-30 cm) was 1.3, 1.32 in *Spirulina* and *Trifolium* respectively. The other parameters and all the chemical properties were positively affected under the application of the two treatments either at surface or subsurface soil. Except for exchangeable sodium percentage (ESP) decreased after biostimulants application, ESP at depth (0-15 cm) was 1.83, 1.78 while at depth (15-30 cm) was 1.7, 1.7 in *Spirulina* and *Trifolium* respectively and this proved that there is an inverse relationship between ESP and Soil Organic Content (SOC). The use of biostimulants led to rising soil PH from acidic in the surface soil (6.8) to slightly alkaline (7.6) and was also observed in subsurface soil. Percent of clay carbonate increased in treatments, especially in the surface soil. Moreover, clay carbonate in the subsurface soil either in the two treatments was less than surface soil.

**Table 3.** yield components under effect of different concentrations of *Trifolium alexandrinum* and *Spirulina platensis*

| Fertilizers (g) | | Components | | | |
|---|---|---|---|---|---|
| ***T. alexandrinum*** | ***S. platensis*** | **No. of pods plant$^{-1}$** | **Pod length (cm)** | **No. of seeds pod$^{-1}$** | **Dry wt. of 100 seeds (g)** |
| 0 | 2.5 | 4.33±0.47*** | 5.5 ±0.50** | 8.5±0.5** | 13.5 ± 0.50** |
| 0 | 5 | 5 ± 0.82*** | 6.6±0.60** | 9.5±0.5** | 17.5±0.50*** |
| 0 | 10 | 5.6 ±0.82*** | 8.1±0.10*** | 10.5 ±0.5** | 21.5±0.50*** |
| 2.5 | 0 | 6.5±0.5*** | 9.7±0.7** | 9.5 ±0.5** | 17.5±0.50*** |
| 5 | 0 | 7.5 ±0.5*** | 10±0.50*** | 10 ± 0.00** | 19 ±1.00** |
| 10 | 0 | 7.8 ± 0.5*** | 10.55±0.55*** | 10.5±0.5** | 19.5±0.5*** |
| 2.5 | 2.5 | 5.33± 0.00*** | 9.17±0.17*** | 10.5±0.5** | 19±1.00** |
| 5 | 2.5 | 6± 1.00*** | 9.5±0.50*** | 11.5±0.5** | 19.5±0.50*** |
| 10 | 2.5 | 6 ±1.00*** | 9.6±0.60*** | 11.5±0.5** | 20.5±0.50*** |
| 2.5 | 5 | 5.5±0.5*** | 10.2±0.20*** | 11.5± 0.00** | 19.5±1.5** |
| 5 | 5 | 7±0.00*** | 10.5 ± 0.00*** | 11.5 ± 0.5** | 19.66± 1.24** |
| 10 | 5 | 6.5±0.5*** | 10.7 ±0.70** | 12.5 ± 0.5*** | 20.5 ± 0.50*** |
| 2.5 | 10 | 6.66±0.82*** | 10.75±0.25*** | 12 ± 0.00*** | 20.6± 0.47*** |
| 5 | 10 | 7 ±0.81*** | 11.5 ± 0.5*** | 12.5 ± 0.5*** | 20.66± 0.47*** |
| 10 | 10 | 8±0.82*** | 12 ± 0.00*** | 13.5 ± 0.5*** | 21.5 ± 0.5*** |
| | Control | 1.5±0.50 | 3.35±0.00 | 6.5±0.5 | 8.3±0.30 |

Mean± standard errors based on ANOVA analysis, *significant, P<0.05; **significant, P <0.01; ***significant, P<0.001 and Ns is nonsignificant difference

**Table 4.** Physical and chemical characteristics of soil incorporated with the highest concentrations of *Trifolium alexandrinum* (T) and *Spirulina platensis* (S)

| Characteristics | Depth (0-15 cm) | | | Depth (15-30 cm) | | |
|---|---|---|---|---|---|---|
| | **Control** | **S (10 g)** | **T (10 g)** | **Control** | **S (10 g)** | **T (10 g)** |
| Sand (2-0.05) | 5.6 | 3.87 | 4 | 6.2 | 4.21 | 4.8 |
| Silt (0.05-0.002) | 35 | 36.5 | 36.5 | 34 | 34.6 | 34.6 |
| Clay (<0.002) | 59.4 | 60.5 | 60.2 | 59.8 | 59.7 | 59.4 |
| BD (mgm$^{-3}$) | 1.2 | 1.43 | 1.4 | 1.2 | 1.4 | 1.4 |
| COLE | 1 | 1.34 | 1.36 | 1 | 1.3 | 1.32 |
| HC (cmhr$^{-1}$) | 1.9 | 3 | 2.8 | 2.9 | 3.4 | 3.2 |
| WDC% | 4.6 | 9.1 | 9 | 3.8 | 9.8 | 9.8 |
| pH | 7 | 7.8 | 8 | 8 | 8.1 | 9 |
| EC dsm$^{-1}$ | 0.2 | 0.35 | 0.31 | 0.2 | 0.32 | 0.28 |
| % SOC | 0.76 | 1.08 | 1.04 | 0.86 | 1.03 | 0.99 |
| % $Caco_3$ | 9.6 | 14 | 13.5 | 11.9 | 13.4 | 12.9 |
| %clay carbonate | 3.8 | 4.88 | 4.56 | 2.6 | 3.92 | 3.6 |
| CEC | 47.1 | 61 | 59 | 48.3 | 61.6 | 59.6 |
| Clay CEC | 87 | 95 | 95 | 90 | 99 | 99 |
| %BS | 74 | 76.3 | 75.5 | 71 | 71.8 | 71 |
| ESP | 2 | 1.83 | 1.78 | 1.8 | 1.7 | 1.7 |
| **S.D.** | 1.40 | 2.03 | 1.87 | 1.41 | 2.21 | 1.37 |
| **P-value** | 0.003 | 000 | 000 | 0.002 | 000 | 0.001 |
| **F-test** | ** | *** | *** | ** | *** | ** |

Abbreviations: BD: Bulk Density, BS: Black Soil, CEC: Cation Exchange Capacity, COLE: Coefficient of Linear Extensibility, ESP: Exchangeable Sodium Percentage, HC: Hydraulic Conductivity, SOC: Soil Organic Content, WDC: Water Dispersible Clay.

### *Nutrient status of soil*

With respect to soil element contents (Table 5) including, Extractable bases (Ca, Mg, Na and K), macronutrients (N, P, K, S, Ca and Mg) and micronutrients (Cu, Mn, Fe, Zn, B and Se). The results showed highly significant increases in all tested elements more than the corresponding control.

Also, these contents in subsurface soil were higher than the surface soil.

**Table 5.** Element contents of soil incorporated with the highest concentrations of *Trifolium alexandrinum* (T) and *Spirulina platensis* (S)

| Elements | Depth (0-15 cm) | | | Depth (15-30 cm) | | |
|---|---|---|---|---|---|---|
| | Control | S (10 g) | T (10 g) | Control | S (10 g) | T (10 g) |
| | | | **Extractable bases (mmolc L$^{-1}$)** | | | |
| Ca | 36.2±0.33$^{e}$ | 40.2±0.57$^{d}$ | 40.5±0.59$^{c}$ | 40.3±0.56$^{d}$ | 41±0.8$^{c}$ | 41.3±0.46$^{b}$ |
| Mg | 6.2±0.19$^{d}$ | 14.06±0.49$^{a}$ | 13.4±0.5$^{b}$ | 11.9±0.15$^{c}$ | 13.56±0.41$^{ab}$ | 12.9±0.18$^{b}$ |
| Na | 0.88±0.29$^{d}$ | 2.41±0.19$^{b}$ | 2.66±0.14$^{a}$ | 1.47±0.18$^{c}$ | 2.22±0.1$^{b}$ | 2.47±0.18$^{a}$ |
| K | 0.8±0.01$^{d}$ | 1.2±0.06$^{a}$ | 1±0.14$^{b}$ | 0.74±0.01$^{e}$ | 1.04±0.09$^{b}$ | 0.84±0.03$^{dc}$ |
| | | | **Available macro nutrients (mgkg$^{-1}$)** | | | |
| N | 246.3±0.91$^{d}$ | 262±0.60$^{a}$ | 258.8±0.96$^{b}$ | 225.4±0.73$^{d}$ | 247.4±0.83$^{c}$ | 244.2±0.07$^{a}$ |
| P | 4.99±0.38$^{d}$ | 7.01±0.71$^{c}$ | 6.5±0.46$^{b}$ | 5.34±0.32$^{d}$ | 7.361±1.22$^{c}$ | 6.85±0.86$^{b}$ |
| K | 457.2±0.40$^{e}$ | 582±0.24$^{c}$ | 577±0.20$^{bc}$ | 563.7±0.26$^{d}$ | 688.5±0.28$^{a}$ | 683.5±0.17$^{b}$ |
| | | | **Exchangeable nutrient (mgkg$^{-1}$)** | | | |
| Ca | 35.6±0.04$^{c}$ | 39.3±0.03$^{a}$ | 37.4±0.04$^{b}$ | 27.2±0.02$^{e}$ | 30.9±0.03$^{bc}$ | 29±0.01$^{cd}$ |
| Mg | 17.1± 0.32$^{c}$ | 18.9±1.52$^{b}$ | 18.08±1.38$^{b}$ | 14.2± 1.13$^{c}$ | 16± 0.52$^{a}$ | 15.18± 0.50$^{ab}$ |
| S | 22.5± 0.74$^{c}$ | 30± 1.13$^{a}$ | 28± 0.46$^{ab}$ | 19.2± 0.49$^{c}$ | 26.7± 0.52$^{b}$ | 29.7± 1.10$^{a}$ |
| | | | **Available micro nutrients (mgkg$^{-1}$)** | | | |
| Cu | 2.23±0.06$^{e}$ | 4.08±0.12$^{a}$ | 3.02±0.11$^{d}$ | 2.75±0.07$^{dc}$ | 3.56±0.13$^{b}$ | 3.54±0.05$^{c}$ |
| Mn | 5.88±0.35$^{c}$ | 6.91±0.46$^{a}$ | 6.67±0.42$^{ab}$ | 3.65±0.35$^{e}$ | 4.68±0.37$^{b}$ | 4.44±0.91$^{b}$ |
| Zn | 0.99±0.32$^{c}$ | 1.35±0.37$^{a}$ | 1.22±0.38$^{b}$ | 1.06±0.42$^{c}$ | 1.42±0.34$^{a}$ | 1.29±0.29$^{b}$ |
| Fe | 2.99±0.38$^{e}$ | 4.4±0.39$^{b}$ | 4±0.21$^{c}$ | 3.85±0.32$^{d}$ | 5.26±0.53$^{a}$ | 4.86±0.40$^{ab}$ |
| B | 0.25±0.33$^{c}$ | 0.48±0.29$^{a}$ | 0.43±0.36$^{b}$ | 0.07±0.06$^{e}$ | 0.3±0.98$^{bc}$ | 0.25±0.3$^{c}$ |
| Se | 8.45±0.34$^{d}$ | 9.65±0.65$^{b}$ | 10±0.40$^{a}$ | 7.84±0.27$^{e}$ | 10±0.41$^{a}$ | 9.39±0.31$^{c}$ |

Mean± standard error based on ANOVA analysis. Means in the same raw followed by the same letter in each column are not significantly different from each other at the 5-percent probability level (p value at 0.05) according to Duncan Multiple Range Test (DMRT).

## Discussion

*Spirulina platensis* and *Trifolium alexandrinum* were chosen to achieve the goal of this study depending on their chemical constituents recorded in (Table 1). Vegetative growth characteristics of pea were positively affected by biostimulants application. This finding could be explained by the fact that biostimulants affect the plant roots structure on the same line of IAA, triggering a "nutrient acquisition response" which favors nutrient uptake by increasing absorptive surface area (24). Also, they enhance nutrient use efficiency, stimulate plant development and eventually enhance crop quality (25). This is likely resulting in an increase in plant biomass (26). The positive effect of *Spirulina sp.* on plant growth may be attributed to the production of phytohormones like auxins and gibberellin which increase cell division and development (27). Furthermore, It is known that *Trifolium* contains high amounts of trace elements like Cu, Fe, Se, Mn and Zn which are important in cell division and enlargement as well as photosynthesis resulting in increased shoot growth (28).

The yield components of pea were significantly affected by the application of biostimulants, our results are consistent with (29) who claimed that fertilized pea significantly skipped the unfertilized ones in all yield components such as weight, length as well as the number of seeds and pods. Additionally, it was clarified that the application of biostimulants led to a significant increase in the number of pods and seeds (30). This increase may be due to biostimulants adding organic matter to the soil consequently improving soil structure and thus enhancing plant growth and crop yield (31). Enhanced availability of carbohydrates for seed formation during the flowering stage leads to an increase in seed weights (32), which was likely related to increased root activity for uptake of nutrient efficiency (33). By the way, *Trifolium* as green manure, when intercropped with oats in maize, increased maize yield by 10% and returned 43 kg N ha$^{-1}$ (34). The beneficial role of *Trifolium* in plant growth results in higher yield due to enhance photosynthetic activity by N fixation and is consistent with (35).

Most parameters of physical and chemical properties of soil were positively affected under the application of the two bio treatments either at surface or subsurface soil. Except for exchangeable sodium percentage (ESP) decreased after biostimulants application and this proved that there is an inverse relationship between ESP and Soil Organic Content (SOC). Results showed no change in water dispersible clay (WDC) and This agrees with (36), WDC is a good predictor of water-induced soil erosion and subsequent nutrient and pollution losses. It was stated that HC is impaired in soil with increasing contents of calcium carbonate and ESP (37). Moreover, the soil appears sequestering more inorganic carbon will have highly impaired HC value (38). By the way, bulk density indicates the weight of all the organic and inorganic materials of a given volume of soil. High clay content and introduction of farm machinery cause compaction of surface layer, which increases BD. The calcium carbonate present as a chemical compound helps in binding soil particles, causing a greater cohesion between soil particles. This leads to greater compaction that increases BD values this agrees with (39). Organic carbon requisition addressed in the soils with high ESP and low HC. There is a positive correlation between HC and SOC since it is known that the increase in SOC solubilizes native $CaCO_3$ (40) which would, in turn, decrease the ESP/SAR (Sodium adsorption ratio) and increase the HC of soils (41). Also, HC increased with decreasing in SIC which attributed to a decrease in ESP (42). Linear Extensibility (LE) is a tool for predicting how much soil can shrink and swell. The LE of a soil layer is equal to the product of the layer's thickness in centimeters multiplied by the layer's COLE. The LE of a soil is the sum of these products for all soil horizons. A positive link between LE and smectite content can occur since greater COLE values suggest the existence of more shrink-swell minerals, such as smectite (43).

The chemical analysis of soil, after the application of two biostimulants, changed soil PH from acidic in surface soil (6.8) to slightly alkaline (7.6). However, the same trend was also observed in subsurface soil, these results agree with (44) stated that the formation of calcium carbonate is a basic process for the development of sodality and increase in soil pH. In the case of Calcium carbonates contents in soils treated either by *Spirulina* or *Trifolium*, data showed that % of $CaCO_3$ was generally higher than control. It is proved that an increase in SIC is widely recognized to enhance a soil system's ESP (42). Therefore, the formation of calcium carbonate is also a basic process for the development of sodality (44) and this led to an increase in soil pH. Moreover, % of clay carbonate increased in treatments, especially in the surface soil. Also, clay carbonate in the subsurface soil either in the two treatments was less than surface soil. From the data in (Table 4), we can deduce that there is a reversible relationship between SOC and clay carbonate. Generally, the results of soil analysis indicate that there is an enhancement in soil fertility and this may be due to Legume contributing to adding atmospheric N to the soil through bacterial N fixation (45).

With respect to the element contents in soil, either macro or micronutrients in subsurface soil were higher than the surface soil. These results are consistent with a previous study by (46) where chemical transformations of soils and availability of major nutrients such as nitrogen, phosphorus, potassium and sulfur are enhanced by using biostimulants. The use of *Trifolium* biostimulant, which made these nutrients more available in the soil, may have increased micronutrient concentrations. Moreover, a recent study showed that the inoculation of cyanobacteria could also increase the availability of macronutrients (C, N, P and K) in soil and their translocation inside plants (47). *Trifolium* is also an N-fixing legume that requires rhizobium inoculation when grown outside of its native range and reduces erosion by providing soil cover (48). *Spirulina platensis* and *Trifolium alexandrinum*, in general, boost nutrient availability in the soil and increase water and nutrient uptake through the plant root system, which is consistent with previous research (49).

## Conclusion

This study focuses on the potential use of two types of biostimulants, *Spirulina platensis* and *Trifolium alexandrinum* in sustainable agriculture. Vegetable crops are the primary food supply in underdeveloped countries, hence they are regarded as the main source of income for the rural population. However, agricultural productivity is highly dependent on soil fertility. Both biostimulants are significant not just in terms of their potential impacts on soil fertility, but also in terms of pea plant development and growth. Whereas biostimulants effectively release phytohormones, macro and micronutrients which improve soil fertility, regulate plant growth and increase target activity. The findings of this study reveal that a single biostimulant had a good effect on pea plants when compared to the control, with the combination of two fertilizers resulting in even more improvements. Additionally, biostimulants improve soil criteria and increase mineral availability leading to stimulations of plant growth characteristics and yield. Overall, our data imply that biostimulants can be used effectively and safely in agricultural applications.

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## Authors contributions

ASS and FGK Conceptualization. AG Methodology and investigation. SAI Formal analysis and Data curation. SAI Writing, Review and editing. ASS and FGK Supervision. All authors have read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest**: Authors do not have any conflict of interests to declare.

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