Full length article

Title **"Sewage sludge and phytohormones combined effect on growth and antioxidants of three broad bean cultivars"**

Running title **"Sewage sludge and phytohormones effect on three broad bean cultivars"**

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**Novelty Statement**

* This work discusses the response of different broad bean cultivars to different concentrations of sewage sludge (SS) added to the cultivated soil as a potentially beneficial fertilizer on both economic and ecological aspects. It also tests the effect of spraying treated plants by IAA and SA to record any potential synergism in plant growth stimulation or alleviation of possible negative SS effects on plant growth. This combined effect has not been previously discussed in literature.
* The results revealed that broad bean cultivars did not respond similarly to sewage sludge treatment, particularly high SS concentrations, therefore, cultivar selection is crucial.
* According to this work, the success of broad bean cultivar in enhancing its growth under SS treatment is related to an active antioxidant system, and vice versa.
* Phytohormonal spraying by IAA or SA can alleviate the toxic impacts of SS treatment.
* The findings of this article highlight the essentiality of cultivar selection when using SS as a fertilizer. Appropriate cultivar selection can significantly increase crop production and therefore, encourages the use of SS as a fertilizer which will be of great impact both economically to reduce the cost of chemical fertilizers and environmentally in the course of nutrients recycling.

**Abstract**

Adding Sewage sludge (SS) to cultivated soil affects physical and chemical soil characteristics. SS can be used as a beneficial fertilizer due to its high contents of organic matter, macro and micronutrients. It also decreases soil pH which affects the availability and absorption of several nutrients and affects soil microbial communities. However, SS can also be toxic to cultivated crops, particularly due to its high heavy metals content and altering nutrients availability. In this work, three broad bean cultivars were cultivated in different SS levels to evaluate and compare their responses for recording to what extent their responses might differ. The response of treated plants to spraying by IAA and SA hormones was also studied. Lengths of roots and stems, no. of leaves, leaf area and fresh weights of roots, stems and leaves were measured. Aantioxidant activity by the DPPH assay, contents of ascorbic acid, anthocyanins, free and bound phenolics and flavonoids were assessed. SS treatment stimulated vegetative growth of cultivar Sakha 1 plants up to 1.5-fold that of control plants, while significantly suppressed growth of the other two cultivars Giza 843 and Giza 716. Phytohormonal treatment alleviated the toxic SS effect on cultivars Giza 843 and Giza 716 while inhibited growth of SS-treated Sakha 1 plants. Stimulated growth was accompanied by enhanced antioxidants and vice versa. Therefore, SS can be used as a very beneficial fertilizer for broad bean plants; however, cultivar selection is crucial as cultivars of the same species might respond differently.

**Key words:** Antioxidants; Broad bean; Phytohormones; Sewage sludge

**Introduction**

Sewage sludge (SS) is a term that describes insoluble wastewater biological solid residues or organic wastes resulting from different sewage treatment processes. They are naturally rich in organic carbon, nitrogen, phosphorus and micronutrients, which gives it unique fertilizing benefits. The growing population and urbanization have unavoidably increased the volume of wastewater sludge production. The use of SS is of great benefit for sustainable agriculture management practices, both economically for substituting chemical fertilizers and environmentally for protecting water schemes against SS pollution and nutrients recycling (Elsalam *et al*. 2021). Sludge-amended soil differs, to varying extents, from its equivalent unsludged control soil. It tends to have a higher content of organic matter (OM), a higher concentration of macro-and micro-nutrients, and the activity of soil microorganisms may be different. Adding SS to soil is expected to affect the dissolved organic matter (DOM) which could influence mobility and bioavailability of trace elements in the amended soil as it has functional groups that can form chelates with trace elements forming quite stable complexes (Kosobucki and Buszewski 2011). Thus, agricultural soil amendment with SS can enhance soil physical characteristics and fertility, which ultimately improves crop production (Koutroubas *et al.* 2014).

Many examples of its use on agronomic crops, forest trees, horticulture crops and plants grown on reclaimed lands have been reported (Larney and Anger 2012; El-Ramady *et al*. 2014; Gubišová *et al*. 2020). Several studies have recorded the positive impact of SS application on legume crops productivity (Chandra *et al*. 2008; Singh and Agrawa 2010; Kumar and Chopra 2014). Antolín *et al*. (2010) indicated a useful effect of SS application for legumes subjected to a cyclic drought. Furthermore, Rebah *et al*. (2007) indicated that several SS constituents are essential to increase the activity of *Rhizobia* and for microbial growth.

However, SS may contain high levels of pollutants, either organic and/or heavy metals (Rastetter and Gerhardt 2017). Therefore, it may lead to environmental pollution if not dealt with appropriately (Eid *et al*. 2017). Heavy metal stress affects the metabolism of most groups of phytohormones (Piotrowska‐Niczyporuk *et al*. 2020). External supplementation of phytohormones can help recover the natural levels of these hormones. Salicylic acid has a protective role against heavy metal stress through induction of phytochelatin synthesis (Szalai *et al*. 2013). The effective role of auxins in heavy metal mitigation was also demonstrated (Nguyen *et al*. 2021). Broad bean is the third most cultivated legume crop worldwide and is grown in more than sixty countries (Abdel Latef *et al.* 2021).

The aim of the present work was to compare the impact of different SS levels on growth and antioxidants of three different broad bean cultivars and to evaluate the impact of spraying plants under different treatments by the phytohormones IAA and SA to record any possible role in the alleviation of potential toxicity of toxic SS levels or enhancement of growth stimulation under fertilizing SS levels.

**Materials and Methods**

A pot experiment was designed at the Botanical Garden of the Faculty of Science, Minia University starting from October 2020. Control soil was obtained from Minia University Botanical Garden while dewatered SS was obtained from Almoheet drainage which is a common agricultural and domestic drainage in Minia Governorate. The SS was blended with control soil in rates of 0 (control), 20%, 50%, 70% and 100% SS. Each pot was filled with 4 kg of the respective mixture. Broad bean seeds of three different cultivars (Sakha 1, Giza 843 and Giza 716) were obtained from Faculty of Agriculture, Assiut University, Egypt. Seeds were surface sterilized using a hydrogen peroxide ethanol mixture (1:1 v/v) and washed thoroughly by distilled water. Five seeds were sown per pot and the seedlings were thinned after 10 days of germination to three per pot. After 4 weeks, three replicates of each SS level treatment were sprayed by IAA (200 ppm) and three other replicates were sprayed by SA (10 mM). Two weeks later, whole plants were collected and washed thoroughly by distilled water. Fresh weights of roots, stems and leaves were determined as well as root length, stem length and number of leaves per plant. Average leaf area was determined according to the disk method (Watson and Watson 1953). Fresh plant leaves were frozen at -25º for analysis. For analyses that required dried tissues, samples of plant leaves were dried at 70ºC for 48 hours until a constant weight was reached and were then ground into fine powder and kept in paper bags for analysis. Plants under control, 50% and 100% SS levels were analyzed for their contents of antioxidants.

**Soil Analysis**

Samples of both control soil and SS were air-dried, ground, passed through a 2 mm sieve and kept for analysis. Soil pH by pH-meter was measured in 1:2.5 soil-water suspension after shaking for 30 minutes for extraction. Electrical conductivity (EC, dS m-1) was measured in 1:1 soil-water extract using a conductivity meter. Organic matter (OM) was estimated in both soil and SS by the loss on ignition method (Cuniff 1997).The total content of macronutrients were estimated by titration while contents of heavy metals were determined after digesting the samples in a mixture (3:2 v/v) of 70% HClO4 and 65% HNO3, and analyzing the filtrates using atomic emission spectrometer (PerkinElmer AAnalyst 400) (Shi *et al*. 2019).

**DPPH Assay**

A weight of 0.05 g of dried powdered plant leaves was extracted in 5 mL of 50% methanol overnight, then centrifuged at 14000 rpm for 15 min and the supernatants were used for both DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and determining total flavonoids content (Tusevski *et al.* 2014) .

Free radical scavenging activity was measured using DPPH (McCune and Jones 2001). One mL of 0.1 mM DPPH in methanol was mixed with 1 mL of methanol extract of each sample, incubated in dark for 30 min and absorbance at 517 nm was read. The DPPH solution without sample exhibits a deep purple color with an absorption maximum at 517 nm and was used as control while the 50% methanol was used as a blank. The purple color disappears when an antioxidant is present in the medium, resulting in a decrease in absorbance. The percentage scavenging activity was calculated using the following equation:

% SA = [(A0 - A1)/ A0] × 100

Where % SA= percentage scavenging activity, A0 = absorbance of control, A1 = absorbance of sample. The higher % SA indicates higher antioxidant activity.

**Determination of Ascorbic Acid Content**

Ascorbic acid content was estimated after Jagota and Dani (1982) where 0.1 g of fresh plant leaves was homogenized with 2 mL of 0.75 M metaphosphoric acid, centrifuged for 10 min at 3000 rpm and 200 μL of 3% metaphosphoric acid and 200 μL of Folin reagent (1:5) were added to 400 μL of the supernatant and brought to a total volume of 2 mL with distilled water. After 10 min of mixing, the absorbance was read at 760 nm. The ascorbic acid contents were calculated using a standard curve constructed with known concentrations of ascorbic acid (50-1000 ug mL-1).

**Determination of Total Anthocyanins Content**

A sample of 0.1 g of fresh frozen leaves was ground in liquid nitrogen and extracted in 2 mL of 1% HCl acidified methanol (1% (v:v) HCl in methanol) for 2 h at room temperature (20-25°C). Samples were centrifuged for 15 min at 12000 rpm and absorbance of supernatant was read at 535 nm against acidified methanol as a blank (Strack and Wray 1989). To calculate anthocyanins concentration, the dilution factor and cyanidin 3-galactoside coefficient of extinction (98.2) were used (Lees and Francis 1972) according to the equation:

Total anthocyanins (mg cyaniding g-1 fresh plant material) = (Absorbance x dilution factor)/ 98.2

**Determination of Free and Wall-bound Phenolic Compounds**

A weight of 0.25 g fresh leaves was extracted in 5 mL of 50% methanol (1:1 v/v) for 90 min at 80°C, centrifuged for 15 min at 14000 rpm and the supernatant was collected for determining free phenolics. The pellet was further mixed with 2 mL of 0.5 N NaOH for 24 h at room temperature (20-25°C) to release bound phenolics, neutralized with 0.5 mL of 2 N HCl, centrifuged for 15 min at 14000 rpm and the supernatant was used for estimating bound phenolics (Kofalvi and Nassuth 1995).

Free and bound phenolics were determined using Folin Ciocaltaeu method. Of each sample, 100 µL was diluted to 1 mL with deionized water and mixed with 0.5 mL of 1 N Folin Ciocalteau reagent (1:1 diluted commercial 2N reagent). After 5 minutes, 2.5 mL of 20% Na2CO3 was added to each sample. Samples were incubated for 20 min at room temperature and absorbance measured at 735 nm against a blank. A standard curve was constructed with gallic acid of known concentrations (100-1000 μg mL-1) prepared in 50% methanol. Total phenolic content was expressed as mg gallic acid equivalents (GAE) g-1 fresh leaves.

**Determination of Total Flavonoids Content**

Five hundred μL of 2% AlCl3 in methanol was added to 0.5 mL of samples extracted in methanol. After 1 h incubation at room temperature (20-25°C), the absorbance was measured at 420 nm. A standard curve was prepared using quercetin of known concentrations (20-200 μg mL-1) prepared in 50% methanol. Total flavonoids content was expressed as mg quercetin equivalents (QE) g-1 dried plant material (Ordonez *et al.* 2006).

**Statistical analysis**

All data were subjected to one-way analysis of variance (ANOVA) using SPSS (ver. 21.0) and represented as means of three replicates ± SE. Least significant difference (L.S.D) was used to compare means ants at 0.05 significance level.

**Results**

Table 1 shows the results of soil analysis for both control soil and 100% SS and indicate decreased pH and increased organic matter and contents of different minerals in SS compared to control soil. The results of different growth parameters (fresh weights of roots, stems and leaves, root length, number of leaves and leaf area) under different treatments are represented in tables 2, 3 and 4 for cultivars Sakha 1, Giza 843 and Giza 716, respectively. For cv. Sakha 1 (table 2), almost all estimated growth parameters increased by increasing SS level in the cultivated soil. The best growth for this cultivar was recorded at 70% SS level. For example, root, stem and leaves fresh weights recorded 4.67g, 12.6g and 10.6g, respectively at 70% SS level compared to 3.09g, 8.62g and 6.14g, respectively in control plants. Spraying plants of this cultivar by IAA under the same SS levels enhanced root length, stem length, number of leaves and leaf area of the tested plants under most tested SS levels while the other growth parameters decreased with IAA application. All growth criteria of this cultivar under combined SS and SA application decreased in comparison to control.

Table 3 represents the measured growth parameters of cultivar Giza 843, all significantly decreased by increasing SS level in cultivated soil compared to control plants. The highest growth reduction was recorded in 100% SS cultivated plants. Some growth parameters recorded less than 50% of control values in 100% SS cultivated plants of this cultivar. For example, root and leaves fresh weights recorded 1.00g and 4.05g, respectively in 100% SS treated plants compared to 2.05g and 9.66g, respectively in control plants. IAA spraying of SS treated plants resulted in slight alleviation of the SS negative impact on growth of this cultivar. Growth parameters of IAA+SS treated plants are higher than their corresponding SS treated plants without IAA spraying. Salicylic acid spraying also stimulated most growth parameters of SS treated plants in comparison with non-sprayed plants, with the exception of stem length and stem fresh weight.

Table 4 represents growth criteria of tested broad bean cultivar Giza 716 which also shows highly significant growth reduction with increased SS level in cultivated soil. Only root length of SS treated plants of this cultivar increased with increasing SS level. Phytohormonal spraying of SS treated plants, either with IAA or SA, resulted in increased growth parameters of sprayed SS treated plants in comparison with their corresponding non-sprayed SS treated plants. Also root length was the only exception in hormonal treated plants as it recorded lower values in IAA and SA treated plants compared to plants which did not receive hormonal treatment.

The data of antioxidant activity and different antioxidants are recorded in tables 5, 6 and 7 for cultivars Sakha 1, Giza 843 and Giza 716, respectively. Table 5 representing antioxidants of cultivar Sakha 1 shows stimulation in antioxidant activity and almost all tested antioxidants in response to SS treatment, particularly in the highest SS level. SS treated plants sprayed by IAA and SA recorded decreased values of antioxidants and antioxidant activity in comparison with their corresponding non-sprayed SS treated plants. Antioxidant parameters of cultivar Giza 843 represented in table 6 show reduction in antioxidants and antioxidant activity in response to SS treatment except free phenolic compounds. Plants under combined SS and phytohormones treatment also reduced their contents of antioxidants with increasing SS level; however, the contents of different antioxidants recorded either higher or non-significantly different values in comparison to the corresponding SS treated plants with no hormonal treatment. For example, the antioxidant activity in 50% SS+IAA and 50%SS+SA plants recorded 74.2% and 70.7%, respectively, compared to 61.2% in 50% SS treated plants with no hormonal treatment. Similarly, antioxidants in cultivar Giza 716 plants represented in table 7 show that all measured antioxidants under different SS treatments are either decreased or insignificantly different from control. Compared to SS treated plants without hormonal application, combined hormonal and SS application on plants of this cultivar induced changes in antioxidants and antioxidant activity that are insignificant in most cases.

**Discussion**

The results of this work recorded different responses of the three tested broad bean cultivars towards SS treatment. Cultivar Sakha 1 showed stimulated growth under different SS treatments compared to control plants, particularly 70% SS level. On the other hand, the two cultivars Giza 843 and Giza 716 highly significantly reduced their different growth parameters under all tested SS levels, with the highest SS level resulting in the most growth reduction. Previous studies recorded stimulated growth under SS application. Zeid and Abou El Ghate (2007) showed that seed germination and early growth of bean seedlings were encouraged by irrigation with sewage water. Eid *et al.* (2017) concluded that SS can work as a good organic fertilizer to increase the yield of both cucumber and spinach. Many studies reported the stimulatory effect of low SS levels along with inhibitory effects of high levels of the same SS amendment on the same plants. For example, Kakati *et al.* (2013) showed that a combination of 10% sludge plus 90% farm yard manure presented better seed germination and plant growth of green gram and that 100% sludge presented inhibitory influence and is not appropriate for plant growth as a stimulant. Eid *et al.* (2018) also recorded that SS applications up to 120 t ha−1 caused a significant enhancement in most of the morphological parameters and biomass of broad bean, however, most growth parameters were declined in reaction to 150 t ha−1 SS application. This is similar to the results obtained by the present work which represented different responses to different SS levels and increased growth inhibition under higher doses of SS in all tested cultivars.

SS is known to have fertilizing characters due to their high contents of organic matter as well as macro and micronutrients, and also to have possible toxic influences due to their potential high contents of heavy metals and their effect on soil micro-organisms as well as soil pH which affect soil availability and absorption of several nutrients. Soil pH is documented to decrease on addition of SS amendment to soil, perhaps as a result of the formulation of organic and inorganic acids throughout the decomposition process of SS components under aerobic conditions (Garrido *et al.* 2005; Kumar and Chopra 2014; Eid *et al.* 2018). The SS analysis in the present work is in accordance with these findings as soil OM is significantly higher and soil pH is lower in SS than control soil. SS can display higher or lower HM concentrations depending on its source (Vieira *et al.* 2014). In the present work, heavy metals recorded slight increases in SS compared to control soil as its source is mainly agricultural not industrial drainage.

The inhibition of morphology and biomass of broad bean at higher concentrations of SS-amended soil is probable due to the higher concentrations of HMs in the greater concentrations of SS which influenced the membrane permeability, producing a water stress (Pesci and Reggiani 1992), as well as the high salinity producing high osmotic pressure (Chandra *et al.* 2008). The association between legumes and *Rhizobia* is susceptible to environmental stress like osmotic stress and drought (Zahran 1999).

Antioxidants and antioxidant activity in this study were parallel to growth parameters. SS treatment resulted in growth stimulation in cultivar Sakha 1 along with increased antioxidants; while cultivars Giza 843 and Giza 716 recorded inhibited growth with simultaneous inhibition in most antioxidants under SS treatment. This might refer to the crucial role of antioxidant system in plant responses to SS application.

Although SS treatment stimulated growth of Sakha 1 plants, combined treatment by phytohormones (either IAA or SA) resulted in reduced growth parameters. Growth stimulation of this cultivar under SS application is probably combined with increased endogenous concentrations of phytohormones, therefore, exogenous application of these hormones had inhibiting impact due to the phytohormones concentrations increase beyond stimulatory levels. The opposite situation was recorded in the other two cultivars (Giza 843 and Giza 716) where SS caused growth reduction and combined SS and phytohormones application enhanced growth parameters in comparison to the SS-treated non-sprayed plants. Most of the measured antioxidants increased on SA spraying of SS-treated plants in the two SS-sensitive cultivars Giza 843 and Giza 716. When a plant is under oxidative stress, one of the main tasks of the defense‐related phytohormone salicylic acid is initiation of the antioxidant system (Ghanta *et al*. 2014).

**Conclusion:**

In conclusion, selection of appropriate cultivars is crucial when using SS in cultivation. SS can be a fertilizer of great benefits both economically and environmentally to broad bean plants. However, it can also be toxic and result in drastic growth reduction dependent on several factors, including SS composition, SS concentration in the cultivated soil and plant cultivar. According to this work, cultivars that are successful in utilizing SS as a fertilizer have an active antioxidant system and vice versa. In the range of this study, Sakha 1 is an appropriate candidate for cultivation in SS-fertilized soil, while Giza 843 and Giza 716 are not. Phytohormonal spraying can be applied to alleviate the negative effects of SS treatment on sensitive cultivars as Giza 843 and Giza 716.

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**Tables**

**Tble 1. Characteristics of control soil and sewage sludge.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Soil | pH | OM (%) | EC | | Cations  (mg kg-1) | | | | Anions  (mg g-1) | | | | Heavy metals (mg kg-1) | | | | |
| dSm-1 | ppm | Ca | Mg | Na | K | Cl | CO3 | HCO3 | SO4 | Cu | Zn | Ni | Fe | Pb |
| Control | **8.16** | **1.4** | **1.06** | **675.2** | **30.07** | **10.86** | **50.06** | **20** | **120.6** | **0** | **230.18** | **50.2** | **1.61** | **6.72** | **6.45** | **6.11** | **2.15** |
| SS | **7.43** | **39.7** | **1.81** | **1157.1** | **40.25** | **30.48** | **90.25** | **22** | **200.16** | **0** | **240.79** | **110.6** | **1.68** | **6.58** | **7.1** | **6.38** | **2.32** |

**Table 2. Effect of different sewage sludge levels (control, 20%, 50%, 70% and 100% SS) and interaction with IAA and SA on stem length (cm), no. of leaves, leaf area (cm2) and fresh weights of roots, stems, and leaves (g) of broad bean cultivar Sakha 1. Values are represented as means of three replicates ± SE, n=3.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| treatment | | Root f.w.  (g) | Stem f.w.  (g) | Leaves f.w.  (g) | Root length  (cm) | Stem length (cm) | No. of leaves | Leaf area (cm2) |
| Control | Control | **3.09±0.14** | **8.62±0.59** | **6.14±0.38** | **35.3±2.7** | **64.3±5.5** | **45±3.7** | **18.41±1.4** |
| 20% SS | **3.28±0.21** | **8.54±0.68** | **6.5±0.49** | **41.2\*±3.2** | **63.3±5.9** | **46.7±3.2** | **19.38±1.6** |
| 50% SS | **3.6\*±0.28** | **10.7\*±0.88** | **7.32\*±0.61** | **40.0\*±3.8** | **61±4.8** | **47.2±4.0** | **19.51\*±1.62** |
| 70% SS | **4.67\*±0.38** | **12.6\*±1.05** | **10.6\*±0.85** | **44.2\*±3.1** | **62±5.6** | **61\*±5.2** | **20.09\*±1.7** |
| 100% SS | **3.59\*±0.27** | **9.12±0.79** | **6.15±0.52** | **43.8\*±4.2** | **54\*±3.0** | **41.3\*±3.2** | **20.21\*±1.8** |
| +IAA | Control | **2.46±0.19** | **7.60±0.56** | **6.27±0.54** | **49.6\*±3.6** | **52\*±3.8** | **64\*±5.5** | **19.96\*±1.7** |
| 20% SS | **2.03\*±0.12** | **7.56±0.6.8** | **5.58\*±0.43** | **41.7\*±2.9** | **57\*±4.2** | **50\*±3.5** | **22.08\*±2.0** |
| 50% SS | **1.99\*±0.12** | **7.14\*±0.61** | **4.92\*±0.28** | **45\*±3.8** | **62±4.8** | **46±3.0** | **17.92±1.4** |
| 70% SS | **1.41\*±0.1** | **6.78\*±0.43** | **4.60\*±0.34** | **49\*±3.1** | **58\*±3.8** | **46±2.8** | **17.18\*±1.5** |
| 100% SS | **1.25\*±0.09** | **5.70\*±0.38** | **4.71\*±0.39** | **50\*±3.8** | **59\*±4.6** | **48±3.1** | **21.45\*±1.8** |
| +SA | Control | **2.04±0.17** | **6.75±0.59** | **4.66\*±0.42** | **39\*±2.2** | **55.5\*±4.2** | **46±4.0** | **19.92\*±1.6** |
| 20% SS | **1.66\*±0.14** | **7.80\*±0.8** | **5.29\*±0.49** | **39\*±2.6** | **62±4.0** | **44.3±2.6** | **18.67±1.2** |
| 50% SS | **1.87\*±0.12** | **9.59\*±0.78** | **3.62\*±0.4** | **41\*±2.9** | **65±3.4** | **43.3±3.8** | **18.34±1.4** |
| 70% SS | **1.0\*±0.08** | **6.30\*±0.54** | **4.12\*±0.36** | **40\*±3.2** | **58\*±3.7** | **46.3±4.0** | **18.26±1.6** |
| 100% SS | **1.15\*±0.08** | **6.13\*±0.62** | **4.01\*±0.28** | **40\*±2.4** | **55\*±2.9** | **47.0±3.5** | **20.88\*±1.7** |

**Table 3. Effect of different sewage sludge levels (control, 20%, 50%, 70% and 100% SS) and interaction with IAA and SA on stem length (cm), no. of leaves, leaf area (cm2) and fresh weights of roots, stems, and leaves (g) of broad bean cultivar Giza 843. Values are represented as means of three replicates ± SE, n=3.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| treatment | | Root f.w.  (g) | Stem f.w.  (g) | Leaves f.w.  (g) | Root length  (cm) | Stem  length (cm) | No. of leaves | Leaf area (cm2) |
| Control | Control | **2.05±0.16** | **13.2±1.02** | **9.66±0.85** | **40.3±2.8** | **77.0±5.8** | **58±3.6** | **16.48±0.98** |
| 20% SS | **1.66\*±0.11** | **9.47\*±0.68** | **6.93\*±0.54** | **42.0±3.9** | **60.3\*±4.3** | **43\*±2.2** | **13.2\*±0.77** |
| 50% SS | **1.51\*±0.12** | **9.97\*±0.74** | **6.89\*±0.58** | **33.3\*±3.1** | **63.7\*±5.2** | **44\*±2.6** | **11.36\*±0.81** |
| 70% SS | **1.31\*±0.11** | **8.26\*±0.69** | **5.32\*±0.47** | **31.8\*±2.5** | **61.3\*±4.7** | **39\*±2.9** | **12.87\*±0.92** |
| 100% SS | **1.00\*±0.08** | **8.17\*±0.58** | **4.05\*±0.28** | **31.5\*±2.7** | **51\*±4.8** | **34\*±3.1** | **13.49\*±1.1** |
| +IAA | Control | **1.62\*±0.12** | **11.3\*±0.96** | **7.15\*±0.66** | **62.7\*±4.5** | **78±5.2** | **50.7\*±3.5** | **17.46\*±1.3** |
| 20% SS | **1.69\*±0.14** | **11.9\*±1.16** | **7.13\*±0.56** | **42.0±3.8** | **73\*±6.0** | **56±4.2** | **15.59\*±1.2** |
| 50% SS | **1.69\*±0.11** | **9.96\*±0.85** | **7.86\*±0.70** | **44.0\*±4.1** | **73\*±6.4** | **66\*±3.8** | **12.01\*±0.85** |
| 70% SS | **1.56\*±0.13** | **10.2\*±0.91** | **6.95\*±0.61** | **48.3\*±2.8** | **65.3\*±5.6** | **52.7\*±2.7** | **11.77\*±0.72** |
| 100% SS | **1.04\*±0.08** | **8.52\*±0.64** | **5.3\*±0.38** | **45.7\*±3.4** | **59.3\*±5.2** | **48\*±3.1** | **8.53\*±0.48** |
| +SA | Control | **2.1±0.18** | **10.6\*±0.86** | **10.0±0.87** | **52.0\*±4.6** | **67\*±5.1** | **50\*±4.1** | **19.21\*±1.47** |
| 20% SS | **1.74\*±0.14** | **7.26\*±0.49** | **6.41\*±0.52** | **51.5\*±3.6** | **62\*±5.7** | **48\*±2.5** | **17.29\*±1.36** |
| 50% SS | **1.81\*±0.15** | **7.35\*±0.62** | **6.99\*±0.64** | **42±2.4** | **55.3\*±3.2** | **58±4.4** | **12.24\*±0.89** |
| 70% SS | **1.3\*±0.1** | **5.75\*±0.47** | **6.01\*±0.47** | **40±3.2** | **56\*±4.4** | **47\*±2.1** | **9.87\*±0.62** |
| 100% SS | **1.41\*±0.11** | **5.81\*±0.55** | **4.68\*±0.38** | **38.0±2.6** | **50\*±3.8** | **49\*±1.9** | **10.07\*±0.59** |

**Table 4. Effect of different sewage sludge levels (control, 20%, 50%, 70% and 100% SS) and interaction with IAA and SA on stem length (cm), no. of leaves, leaf area (cm2) and fresh weights of roots, stems, and leaves (g) of broad bean cultivar Giza 716. Values are represented as means of three replicates ± SE, n=3.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| treatment | | Root f.w.  (g) | Stem f.w.  (g) | Leaves f.w.  (g) | Root length  (cm) | Stem length (cm) | No. of leaves | Leaf area (cm2) |
| Control | Control | **1.95±0.12** | **12.06±0.97** | **10.2±0.86** | **44.7±2.8** | **72.3±5.5** | **66.3±4.2** | **18.38±1.12** |
| 20% SS | **1.83±0.10** | **8.4\*±0.64** | **7.28\*±0.64** | **45.0±3.2** | **59.3\*±3.8** | **59.3\*±4.7** | **17.07\*±1.14** |
| 50% SS | **1.17\*±0.07** | **7.97\*±0.54** | **5.77\*±0.48** | **61\*±3.7** | **60.6\*±4.4** | **44.3\*±3.2** | **16.16\*±1.3** |
| 70% SS | **1.25\*±0.08** | **7.60\*±0.62** | **5.39\*±0.35** | **58.6\*±3.1** | **58.3\*±4.1** | **44.7\*±2.7** | **11.87\*±0.85** |
| 100% SS | **1.25\*±0.07** | **7.50\*±0.64** | **4.96\*±0.37** | **59.0\*±4.0** | **62.3\*±5.6** | **47.3\*±3.4** | **11.9\*±0.89** |
| +IAA | Control | **1.75\*±0.11** | **7.44\*±0.56** | **3.58\*±0.28** | **34.7\*±2.2** | **52.7\*±3.9** | **47.3\*±4.0** | **15.3\*±0.13** |
| 20% SS | **1.92±0.15** | **10.64\*±0.85** | **7.86\*±0.52** | **35\*±2.6** | **65.3\*±4.2** | **56.7\*±3.9** | **16.95±0.14** |
| 50% SS | **1.20\*±0.10** | **10.2\*±0.79** | **7.14\*±0.61** | **32\*±2.4** | **60\*±3.8** | **54.7\*±4.2** | **15.52\*±0.12** |
| 70% SS | **1.93±0.16** | **9.76\*±0.72** | **6.42\*±0.45** | **42.3±3.1** | **59.5\*±4.2** | **52.7\*±4.5** | **18.1±0.15** |
| 100% SS | **1.56\*±0.12** | **9.03\*±0.68** | **5.53\*±0.38** | **30.3\*±1.9** | **58.7\*±3.5** | **56.7\*±3.8** | **18.05±0.12** |
| +SA | Control | **1.27\*±0.10** | **8.36\*±0.65** | **5.68\*±0.51** | **27.3\*±1.5** | **62.8\*±4.6** | **52.7\*±2.9** | **22.22\*±0.18** |
| 20% SS | **1.97±0.16** | **10.4\*±0.84** | **7.41\*±0.57** | **32.7\*±2.8** | **66.5±3.2** | **67.3±5.1** | **24.160\*±0.20** |
| 50% SS | **1.78±0.13** | **8.17\*±0.61** | **6.15\*±0.59** | **37.3\*±3.2** | **67.3\*±5.1** | **53.0\*±3.9** | **22.35\*±0.17** |
| 70% SS | **2.5\*±0.17** | **9.45\*±0.82** | **5.92\*±0.44** | **47.5\*±4.1** | **62.5\*±3.8** | **48.0\*±3.4** | **21.94\*±0.16** |
| 100% SS | **1.56\*±0.14** | **8.07\*±0.66** | **5.03\*±0.36** | **54.2\*±4.9** | **50.7\*±3.4** | **52.7\*±4.4** | **15.98\*±0.11** |

**Table 5. Effect of different sewage sludge levels (control, 20%, 50%, 70% and 100% SS) and interaction with IAA and SA on DPPH percentage scavenging activity, total anthocyanins contents (TAC) in mg cyanidine g-1 fresh weight, ascorbic acid content (AAC) in mg g-1 fresh weight, free and wall bound total phenolic compounds (TPC) in mg GAE g-1 fresh weight and total flavonoids content (TFC) in mg QE g-1 dry weight of broad bean cultivar Sakha 1. Values are represented as means of three replicates ± SE, n=3.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| treatment | | Antioxidant activity  (DPPH) | TAC | AAC | TFC | TPC (free) | TPC (bound) |
| Control | Control | **63.1±3.8** | **0.47±0.03** | **29.3±1.8** | **11.7±0.94** | **94.9±5.9** | **124.5±10.1** |
| 20% SS | **66.0\*±4.2** | **0.56\*±0.03** | **30.9±2.2** | **12.6±1.04** | **114.1\*±8.9** | **127.6±10.6** |
| 50% SS | **81.6\*±6.8** | **0.57\*±0.02** | **32.0\*±1.5** | **12.7±1.01** | **126.7\*±10.6** | **144.9\*±11.5** |
| +IAA | 70% SS | **74.9±5.5** | **0.51±0.04** | **21.9±1.6** | **12.3±0.98** | **91.7±7.4** | **68.3±4.3** |
| 100% SS | **64.9\*±4.8** | **0.45±0.02** | **30.0\*±2.2** | **12.4±0.87** | **111.9\*±8.5** | **89.7\*±6.5** |
| Control | **66.6\*±5.2** | **0.45±0.02** | **28.0\*±2.2** | **12.4±0.92** | **84.3\*±5.7** | **111.8\*±12.8** |
| +SA | 20% SS | **71.4±5.9** | **0.50±0.04** | **30.2±2.1** | **12.4±1.02** | **101.6±6.8** | **125.4±11.2** |
| 50% SS | **63.7\*±4.7** | **0.47±0.03** | **34.7\*±2.6** | **12.8±0.89** | **106.9\*±7.5** | **114.1\*±13.4** |
| 70% SS | **60.3\*±5.1** | **0.39\*±0.03** | **30.2±1.9** | **12.3±1.05** | **123.5\*±9.8** | **97.1\*±7.8** |

**Table 6. Effect of different sewage sludge levels (control, 20%, 50%, 70% and 100% SS) and interaction with IAA and SA on DPPH percentage scavenging activity, total anthocyanins contents (TAC) in mg cyanidine g-1 fresh weight, ascorbic acid content (AAC) in mg g-1 fresh weight, free and wall bound total phenolic compounds (TPC) in mg GAE g-1 fresh weight and total flavonoids content (TFC) in mg QE g-1 dry weight of broad bean cultivar Giza 843. Values are represented as means of three replicates ± SE, n=3.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| treatment | | Antioxidant activity  (DPPH) | TAC | AAC | TFC | TPC (free) | TPC (bound) |
| Control | Control | **75.9±2.1** | **0.59±0.03** | **27.7±1.9** | **12.5±0.97** | **89.46±3.7** | **138.7±7.4** |
| 50% SS | **61.2\*±1.8** | **0.49\*±0.02** | **22.1\*±1.6** | **12.5±0.88** | **106.94\*±3.5** | **129.1\*±6.9** |
| 100% SS | **66.9\*±2.4** | **0.38\*±0.03** | **24.8\*±1.8** | **12.4±1.01** | **99.6\*±4.2** | **121.3\*±8.5** |
| +IAA | Control | **75.7±3.1** | **0.56±0.04** | **31.5±2.1** | **13.4±1.1** | **90.7±5.8** | **112.3±7.8** |
| 50% SS | **74.2\*±2.7** | **0.50\*±0.05** | **28.0\*±2.2** | **11.7\*±0.89** | **103.9\*±4.4** | **149.8\*±9.2** |
| 100% SS | **69.7±1.9** | **0.37\*±0.03** | **29.9±1.9** | **11.9\*±0.95** | **99.21\*±4.9** | **144.2\*±8.8** |
| +SA | Control | **73.5±2.7** | **0.45±0.03** | **34.4±2.6** | **12.2±0.98** | **90.3±3.8** | **162.7±11.5** |
| 50% SS | **70.7±2.5** | **0.43±0.02** | **36.8±2.2** | **12.1±1.05** | **116.3\*±4.1** | **170.1\*±12.4** |
| 100% SS | **69.4±2.4** | **0.38\*±0.04** | **30.5\*±2.7** | **11.9±0.79** | **98.9±3.5** | **199.5\*±14.7** |

**Table 7. Effect of different sewage sludge levels (control, 20%, 50%, 70% and 100% SS) and interaction with IAA and SA on DPPH percentage scavenging activity, total anthocyanins contents (TAC) in mg cyanidine g-1 fresh weight, ascorbic acid content (AAC) in mg g-1 fresh weight, free and wall bound total phenolic compounds (TPC) in mg GAE g-1 fresh weight and total flavonoids content (TFC) in mg QE g-1 dry weight of broad bean cultivar Giza 716. Values are represented as means of three replicates ± SE, n=3.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| treatment | | Antioxidant activity  (DPPH) | TAC | AAC | TFC | TPC (free) | TPC (bound) |
| Control | Control | **76.2±4.2** | **0.60±0.03** | **30.5±2.1** | **12.7±0.65** | **94.2±5.2** | **138.5±7.1** |
| 50% SS | **73.8\*±5.3** | **0.52±0.03** | **21.3\*±1.4** | **12.1±0.58** | **86.0\*±4.2** | **107.8\*±5.5** |
| 100% SS | **74.2±5.6** | **0.53±0.04** | **26.2\*±1.8** | **12.2±0.72** | **89.5\*±4.4** | **158.7\*±8.2** |
| +IAA | Control | **81.8±4.8** | **0.58±0.05** | **22.3±1.6** | **11.8±0.71** | **112.6±6.1** | **97.4±4.6** |
| 50% SS | **82.3±3.7** | **0.57±0.04** | **25.7\*±1.9** | **12.1±0.8** | **104.6\*±4.9** | **123.4\*±7.7** |
| 100% SS | **65.6\*±2.9** | **0.57±0.05** | **21.8±1.4** | **12.6±0.69** | **107.0\*±5.1** | **121.3\*±5.9** |
| +SA | Control | **80.5±3.8** | **0.59±0.04** | **35.4±2.4** | **12.6±0.75** | **90.36±4.8** | **150.5±6.9** |
| 50% SS | **63.7\*±2.5** | **0.55±0.03** | **31.6\*±2.1** | **11.4\*±0.56** | **85.7\*±3.2** | **110.9\*±4.8** |
| 100% SS | **67.8\*±3.1** | **0.55±0.03** | **26.3\*±1.8** | **12.6±0.64** | **69.7\*±4.4** | **112.5\*±5.5** |