



Exogenously-applied plant growth biostimulators increase the growth and productivity of *Vicia faba* plants

Mona A.A. Abdel-Kawy¹, Gamal F. Mohamed¹, Hanan A.A. Taie², Mostafa M. Rady¹

¹Botany Department, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt ²Plant Biochemistry Department, National Research Centre, 33 El-Bohouth St. (Former El-Tahrir St.), Dokki 12622, Giza, Egypt

ABSTRACT:

The use of plant growth biostimulators (PGBs), including diluted bee honey solution (BHS), silymarin (Sm) and iodine (I) can improve growth and productivity of crop plants, including faba bean (*Vicia faba* L.). BHS is rich in osmoprotectants, nutrients, vitamins, and many antioxidants making it effective plant growth biostimulator, compared to Sm or iodine, in increasing plant growth and productivity. Therefore, the present study was planned to investigate the potential improvement in leaf chlorophyll content, growth, and pods yield of *V. faba* plants by foliar application of BHS, Sm or iodine by using a completely randomized design. The enhancing impacts of these three PGBs were observed on leaf chlorophyll content, growth, and pods yield of *V. faba* plants. However, BHS (at a rate of 7.5 g L⁻¹) was superior to Sm and iodine mediated further increases in leaf chlorophyll content, growth, and pods yield by 13.4 and 11.8%, 15.7–36.3 and 13.4–22.8%, and 20.0 and 22.7%, respectively. These results suggest the use of 7.5 g bee honey L⁻¹ as a stimulator to increase the growth and productivity of *V. faba* plants.

KEYWORDS: Biostimulators, faba bean, chlorophyll, growth, yield.

1. INTRODUCTION:

Faba bean (*Vicia faba* L.) is a staple leguminous crop, widely grown worldwide. Like dry seeds, fresh pods are consumed by humans around the world due to their high nutritional value (**Semida et al., 2014; Rady et al.,**

2021). Based on dry matter, *V. faba* contains up to 35% protein (Karkanis et al., 2018), 51-68% carbohydrates (Semida et al., 2014), and contains notable amount of essential nutrients (Khazaei and Vandenberg, 2020).

Received:15 /1/2024

-Accepted: 20/2/ 2024 -Published: 28/2/2024

Biostimulants are a promising sustainable strategy to stimulate plant performance under normal or abiotic stress conditions (Rouphael and Colla, 2018; Teklić et al., 2020; Alharby et al., 2021; Rady et al., 2021; Hassan et al., 2023). In this regard, plant growth stimulants, including silymarin (Sm), iodine, and bee honey solution (BHS), all of which contribute to sustainable agriculture and increase crop yields (Semida et al., 2019; Alharby et al., 2021; Rady et al., 2021; Hassan et al., 2021; Rady et al., 2021; Hassan et al., 2023).

Silymarin (Sm) is the primary bioactive substance extracted from all parts of the Silybum marianum (Milk thistle) plant (Ali et al., 2022). Sm comprises flavonolignans and а flavonoid mixture such as silibinin, silvdianin, isosilvchristin, isosilvbin, silychristin, and taxifolin (Marmouzi et al., 2021). Accumulation of Sm in plant tissues highly interacts with environmental stresses, having antioxidant directly properties, detoxifying preventing and ROS inhibiting formation via ROS production to help plants withstand environmental stresses (Ali et al., 2022).

Iodine is an essential nutrient for human health and is involved in the synthesis of thyroid hormones, which play a dominant role in plant growth and

metabolism (Medrano-Macías et al., 2016; Hassan et al., 2023). There is no enough information available on the influence of iodine on plant morphology, physiology, biochemical characteristics, and plant productivity as iodine is not essential for plants. Thus, little papers have explored that iodine positively influences plant growth and productivity (Smoleń et al., 2015; Medrano-Macías et al., 2016; Hassan et al., 2023).

BHS is a natural biostimulant that mainly contains monosaccharides, disaccharides, and oligosaccharides (Shin and Ustunol, 2005; Saxena and **2010).** It also Gautam, contains various substances such as minerals, enzymes, proteins, lipids, organic acids, inorganic acids. and phenolic compounds (phenolic acids, flavonoids) (Saxena and Gautam, 2010; Inés et al., 2011). BHS serves as an active antioxidant in scavenging ROS (Semida et al., 2019; Rady et al., 2021) due to the presence of flavonoids that inhibit auto-oxidation (Inés et al., 2011) and enzymes that contribute to the removal of ROS (Saxena and Gautam, 2010) to effectively protect against LiW-induced oxidative damage. As reported by Semida et al. (2019) and Rady et al. (2021), BHS, as a plant biostimulator, can increase tolerance to abiotic stress in plants. A field study highlighted the ability of BHS-based plant biostimulants to alleviate salt stress in onions (Semida et al., 2019). Indeed, BHS applied to onion leaves showed higher biomass production, bulb vield, WUE, and leaf photosynthetic pigment contents. Moreover, BHS promoted both enzymatic and non-enzymatic antioxidants, membrane integrity, and water content in onion tissues under the influence of salt stress.

However, little work has been conducted on exogenous application of Sm, iodine, or HBS, as plant growth biostimulants (PGBs), to improve plant performance. Therefore, the current study was planned to evaluate the possibility of using Sm, iodine, or HBS as promising tools to increase *V. faba* performance. This research is designed to examine potential positive changes in growth and pod yield of *V. faba* plants and foliar spraying with Sm, iodine, or HBS.

2. MATERIALS AND METHODS:

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2.1. Experimental Location and Soil Properties:

Pot experiments were conducted in the 2022 season. One hundred and fifty pots (diameter 40 cm, depth 35 cm) were used in this study. All pots were filled with clay loam soil. Soil physicochemical properties were performed using the methods of Klute (1986) and Page et al. (1982), and the results are shown in Table 1. The electrical conductivity of the tested soil was 2.28 dS m⁻¹, being normal soil according to the classification of Dahnke and Whitney (1988).

Table 1. The analysis of physicochemical properties of the experimental soil (presented as averages across both seasons)

| Property | Unit | Value | | |
|--|---------------------------------------|-------|--|--|
| Physical: | | | | |
| Particle size distribution: | | | | |
| Sand | | 15.50 | | |
| Silt | (%) | 16.20 | | |
| Clay | | 68.30 | | |
| Texture class | Clay | loam | | |
| Bulk density | g cm ⁻³ | 1.44 | | |
| Hydraulic conductivity (K _{sat}) | $(\operatorname{cm} h^{-1})$ | 2.99 | | |
| Field capacity | | 32.45 | | |
| Wilting point | (%) | 26.65 | | |
| Available water | | 17.79 | | |
| Chemical: | | | | |
| pH [soil water (w/v) ratio of 1:2.5] | | 7.34 | | |
| ECe [at a soil - paste extract] | (dS m ⁻¹) | 2.28 | | |
| CEC | (cmol _c kg ⁻¹) | 2.32 | | |
| CaCO ₃ | | 4.5 | | |
| Organic matter | mg kg ⁻¹ soil | 0.04 | | |
| Available nutriments | | | | |
| N | g kg ⁻¹ soil | 0.97 | | |
| Р | | 2.32 | | |
| K | mg kg ⁻¹ soil | 54.9 | | |

2.2. Sowing, Treatments, and Experimental layout:

The seeds of *V. faba* (cultivar Giza 716) were secured from the Agricultural Research Center, Egypt. First, the seeds were washed with distilled water and then sterilized with sodium hypochlorite solution (1.0%; v/v) for approximately 2 min. Again,

study, In this 3 separate experiments were conducted simultaneously with one control treatment (e.g., plants were foliar sprayed with distilled water). In the first experiment, plants in 30 pots were foliar sprayed with 3 concentrations of silymarin (Sm) (Table 2), and plants in each 10 pots were assigned to one of the 3 Sm concentrations. In the 2^{nd} experiment, plants in 50 pots were

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the surface of the seeds was cleaned of the sterilization solution with distilled water and then kept at room temperature until dry. The seeds were sown on October 15 at a rate of 5 seeds per pot. Two weeks after sowing, thinning was performed at a rate of 3 plants per pot.

foliar sprayed with 5 concentrations of iodine (I; in potassium iodide) (**Table 2**), and plants in each 10 pots were assigned to one of the 5 iodine concentrations. In the 3^{rd} experiment, plants in 60 pots were foliar sprayed with 6 diluted solutions of bee honey (BHS; raw clover bee honey) (**Table 2**), and plants in each 10 pots were assigned to one of the 6 BHS.

 Table 2. Concentrations of biostimulants (silymarin, potassium iodide, and bee honey) used in this study

| Biostimulants concentrations tested | | | | | | | | |
|-------------------------------------|-------------------------------------|-------|--|---------|---------------------------------|--|--|--|
| | Silymarin | | Potassium iodide | Bee h | oney solution | | | |
| Sm_0 | $0.000 { m ~mg~L^{-1}}$ | Io | $0.00 \text{ mM} = 0.0 \text{ mg } \text{L}^{-1}$ | BHS_0 | $0.0 { m g L^{-1}}$ | | | |
| Sm_1 | $0.200 \text{ mg } \mathrm{L}^{-1}$ | I_1 | $0.05 \text{ mM} = 8.3 \text{ mg } \text{L}^{-1}$ | BHS_1 | 2.5 g L^{-1} | | | |
| Sm_2 | $0.400 \text{ mg } \mathrm{L}^{-1}$ | I_2 | $0.10 \text{ mM} = 16.6 \text{ mg } \text{L}^{-1}$ | BHS_2 | $5.0 \text{ g } \text{L}^{-1}$ | | | |
| Sm_3 | $0.600 \text{ mg } \mathrm{L}^{-1}$ | I_3 | $0.15 \text{ mM} = 24.9 \text{ mg } \text{L}^{-1}$ | BHS_3 | 7.5 g L^{-1} | | | |
| | | I4 | $0.20 \text{ mM} = 33.2 \text{ mg } \text{L}^{-1}$ | BHS_4 | $10.0 \text{ g } \text{L}^{-1}$ | | | |
| | | I_5 | $0.25 \text{ mM} = 38.5 \text{ mg } \text{L}^{-1}$ | BHS_5 | $12.5 \text{ g } \text{L}^{-1}$ | | | |
| | | | | BHS_6 | $15.0 \text{ g } \text{L}^{-1}$ | | | |

All treatments in each experiment completely arranged in a were randomized design. Fifteen days after full emergence of seedling, foliar sprays with Sm and iodine, and BHS concentrations were applied in the early morning. Fifteen days after the first spraying, the 2nd foliar spraying was performed for V. faba plants. Sprays were conducted to run-off, with the use of Tween-20 (0.1%, v/v) as a surfactant to ensure optimum penetration into leaf tissues. In each pot of all experiments, the 3 plants were sprayed with 75 mL of spray solution, which was increased to 100 mL for the second time of spraying. Different fertilizers (40.0 g organic manure, 0.50 g potassium humate, 0.75 kg of P₂O₅ using Ca(H₂PO₄)₂; 15.5% P₂O₅, 0.60 g of K₂O using K₂SO₄; 48% K₂O, and 0.50 g of N using (NH₄)₂SO₄; 21% N were added per pot; 8 kg soil). All agronomic practices were applied

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following the recommendations of the Agricultural Research Center, Giza, Egypt.

2.3. Bee honey analysis:

Clover honey used in this study was analyzed for effective components and results are shown in **Table 3**.

| Table 3. Chemical characteristics of the raw clover be | ee honey (BH) |
|--|---------------|
|--|---------------|

| Components | Units | BH |
|----------------------|------------------------|-----------------|
| Moisture | % | 16.3±0.69 |
| pН | | 4.32±0.21 |
| Total soluble solids | °Brix | 83.7±4.12 |
| Soluble protein | | $0.32{\pm}0.02$ |
| Free amino acids | | $0.33{\pm}0.02$ |
| Total soluble sugars | | 81.2±3.82 |
| Fructose | % | 32.4±2.05 |
| Glucose | <i>~</i> 0 | 39.8±2.84 |
| Sucrose | | 2.45 ± 0.24 |
| Maltose | | 4.53±0.28 |
| Vitamin C | | $0.05{\pm}0.00$ |
| Calcium | | $0.09{\pm}0.00$ |
| Magnesium | | $0.08{\pm}0.00$ |
| Phosphorus | ${ m mg~g^{-1}~FW}$ | $0.07{\pm}0.00$ |
| Potassium | | $0.08{\pm}0.00$ |
| Iodine | | $0.04{\pm}0.00$ |
| Antioxidant activity | mM Trolox eq. L^{-1} | 18.6 ± 0.92 |
| a.: equivalent. | | |

eq.; equivalent.

Moisture (%), total soluble solids, and pH were assessed according to A.O.A.C. (1995). Quantities of sugars High-Performance Liquid bv Chromatography (HPLC) were measured as the concentration of fructose, glucose, maltose and sucrose (%) according to **Bogdanov and** Baumann (1988). Mineral nutrients were measured according to the methodology of Chapman and Pratt (1961). Ascorbic acid concentration was determined according to Mukherjee and Choudhuri (1983). Determination antioxidant of the activity was performed using 1.1diphenyl-2-picrylhydrazyl(DPPH)assay as described by Lee et al. (2003).2.4.Assessment of GrowthCharacteristics and Pods Yield

Forty-five days after sowing, plant growth characteristics were analyzed in each experiment, 5 plants were selected, randomly, from each treatment. Shoot length was measured with a 50-cm ruler. Number of leaves for each plant was counted. Leaf area (cm²) was measured using a held-hand planimeter (Planix 7, Tamaya Technics Inc. Tokyo, Japan). The shoot was weighed for each plant to determine to shoot fresh weight (g). For recording

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shoot dry weight (g), shoots were ovendried at 70 \pm 2 °C until a constant weight was reached. Sixty days after sowing, green pods yield was recorded per pot (3 plants). These parameters of green pods yield were measured using the two outer rows of each experimental plot.

2.5. Determination of Leaf Chlorophyll Content

Leaf total chlorophyll content was determined depending on the method described in Arnon (1949). Homogenization in 80% acetone (v/v) and centrifugation at $10,000 \times$ g for 10 min were implemented. The acetone solution absorption extract was recorded at 663, 645, and 470 nm in a UV-Visible spectrophotometer (UV-160A, Shimadzu, Japan).

2.6. Statistical Analysis

The data were analyzed based on the GLM procedures of the GENSTAT software (VSN International Ltd, Oxford, UK). All data were subjected to the combined analysis and the mean differences were compared with the least significant difference (LSD) test at 5% probability ($p \le 0.05$) level. The analyzed results are presented as the mean \pm standard error.

3. RESULTS AND DISCUSSION:

3.1. Effect of silymarin (Sm) on growth traits and pods yield of *Vicia faba* plants

Table 4 displays that foliar spray with silvmarin (Sm) at all applied concentrations (Sm₁, Sm₂, and Sm₃) increased total chlorophyll (T.Chls) content, growth traits (shoot length; SL, number of leaves per plant; NLP, leaf area per leaf; LAL, plant fresh weight; PFW, and plant dry weight; PDW) and pods yield of Vicia faba plants compared to the control (foliar spray with distilled water; Sm₀). The best concentration was Sm_1 , which increased T.Chls content by 60.5%, SL by 58.8%, NLP by 22.3%, LAL by 43.5%, PFW by 102.8%, PDW by 200.7%, and pods yield by 23.2% compared to Sm₀.

 Table 4. Effect of spraying with silymarin (Sm) on some growth traits and pods yield of Vicia faba plants

| Treatments | T.Chls (mg | Shoot length | Leaves No. | Leaf area | FW | DW | Pods yield |
|---------------------|---------------------|--------------|---------------------|--------------------|--------------------------|--------------------------|------------------------|
| Treatments | g ⁻¹ FW) | (cm) | plant ⁻¹ | (cm ²) | (g plant ⁻¹) | (g plant ⁻¹) | (g pot ⁻¹) |
| Sm ₀ | 1.67±0.08c | 17.0±0.19d | $4.00 \pm 0.00 b$ | 38.2±0.37c | 3.88±0.12c | 1.52±0.08c | 89.4±4.92b |
| Sm_1 | 2.68±0.03a | 27.0±0.33a | 4.89±0.11a | 54.8±0.39a | 7.87±0.23a | 4.57±0.03a | 110.1±5.83a |
| Sm_2 | 2.44±0.37b | 22.1±0.11b | 4.22±0.11b | 46.0±1.12b | $4.87{\pm}0.48b$ | 2.44±0.37b | 95.1±4.76b |
| Sm_3 | 1.98±0.10c | 18.6±0.68c | 4.11±0.11b | 40.6±0.69c | $4.74{\pm}0.05b$ | 1.93±0.10bc | 92.4±3.78b |
| LSD _{0.05} | 0.20 | 1.20 | 0.31 | 2.80 | 0.79 | 0.73 | 2.35 |
| p-value | < 0.0001** | < 0.0001** | < 0.0017** | < 0.0001** | < 0.0001** | < 0.0002** | < 0.0028** |

Mean values within columns followed by the same letter are; ** and * indicate respectively differences at $P \le 0.01$ and $P \le 0.05$ probability level, ^{NS} indicates not significant difference. Means followed by the same letter in each column are not significantly different according to the LSD test (P < 0.05). Total Chls; total chlorophyll content, FW; fresh weight, DW; dry weight, and No.; number.

3.2. Effect of iodine (I) on growth traits and pods yield of *Vicia faba* plants

Table 5 shows that foliar spray with iodine (I) at all applied concentrations $(I_1, I_2, I_3, I_4, and I_5)$ increased plant

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growth traits (SL, NLP, LAL, PFW, and PDW) and pods yield compared to the control (I_0). The best concentration was I_3 , which increased T.Chls content

by 62.9%, SL by 62.4%, NLP by 19.5%, LAL by 46.3%, PFW by 129.1%, PDW by 291.4%, and pods yield by 20.5% compared to I_0 .

Table 5. Effect of spraying with iodine (I) on some growth traits and pods yield of *Vicia faba* plants

| Treatments | T.Chls (mg g ⁻¹ FW) | Shoot length (cm) | Leaves No. plant ⁻¹ | Leaf area (cm ²) | FW (g plant ⁻¹) | DW (g plant ⁻¹) | Pods yield (g pot ⁻¹) |
|-----------------|--------------------------------------|----------------------|-----------------------------------|---------------------------------|--------------------------------|--------------------------------|--------------------------------------|
| I_0 | 1.67±0.08d | 17.0±0.19d | $4.00{\pm}0.00b$ | 38.2±0.37e | 3.48±0.12d | 1.52±0.08e | 89.4±4.92b |
| I_1 | 1.98±0.11c | 23.1±0.62b | $4.33 \pm 0.19b$ | 43.1±0.20c | 4.45±0.13c | 2.37±0.11c | 97.5±4.68b |
| I_2 | 2.34±0.37b | 21.2±0.40c | $4.22 \pm 0.11b$ | 44.8±0.23b | 6.09±0.08b | 3.98±0.09b | 95.1±4.66b |
| I_3 | 2.72±0.03a | 27.6±0.44a | 4.78±0.11a | 55.9±0.29a | 8.89±0.27a | 5.95±0.06a | 107.7±5.71a |
| I_4 | 2.28±0.37b | 18.0±0.58d | $4.00{\pm}0.00b$ | $40.8 \pm 0.82d$ | 4.12±0.03c | 2.01±0.04d | 90.0±3.70b |
| I_5 | 1.96±0.10c | 15.4±0.29e | 3.56±0.11c | 34.8±0.41f | 3.47±0.38d | 1.51±0.19e | 90.3±4.68c |
| $LSD_{0.05}$ | 0.15 | 1.44 | 0.36 | 1.36 | 0.66 | 0.32 | 2.72 |
| <i>p</i> -value | < 0.0001** | < 0.0001** | < 0.0005** | < 0.0001** | < 0.0001** | < 0.0001** | < 0.0035** |

Mean values within columns followed by the same letter are; ** and * indicate respectively differences at $P \le 0.01$ and $P \le 0.05$ probability level, ^{NS} indicates not significant difference. Means followed by the same letter in each column are not significantly different according to the LSD test (P < 0.05). Total Chls; total chlorophyll content, FW; fresh weight, DW; dry weight, and No.; number.

3.3. Effect of bee honey solution (BHS) on growth traits and pods yield of *Vicia faba* plants

Table 6 reveals that foliar spray with bee honey solution (BHS) at all applied concentrations (BHS₁, BHS₂, BHA₃, BHS₄, and BHS₅) increased plant growth traits (SL, NLP, LAL, PFW, and PDW) and pods yield compared to the control (BHS₀). The best concentration was BHS₃, which increased T.Chls content by 82.0%, SL by 90.6%, NLP by 46.8%, LAL by 66.0%, PFW by 143.3%, PDW by 312.6%, and pods yield by 47.8% compared to BHS₀.

Table 6. Effect of spraying with bee honey solution (BHS) on some growth tra its and pods yield of *Vicia faba* plants

| Treatments | T.Chls | Shoot length | Leaves No. | Leaf area | FW | DW | Pods yield |
|---------------------|-------------------------|------------------|---------------------|--------------------|--------------------------|--------------------------|------------------------|
| | (mg g ⁻¹ FW) | (cm) | plant ⁻¹ | (cm ²) | (g plant ⁻¹) | (g plant ⁻¹) | (g pot ⁻¹) |
| BHS ₀ | 1.67±0.08f | 17.0±0.19e | 4.00±0.00b | 38.2±0.37d | 3.88±0.12e | 1.51±0.08d | 89.4±4.92b |
| BHS_1 | 2.28±0.10c | 19.8±0.22d | 4.11±0.11b | 42.3±0.40c | 4.35±0.15d | 2.75±0.15c | 92.4±3.83b |
| BHS_2 | 2.80±0.37b | 21.9±0.48c | 4.33±0.00b | 46.9±0.55b | 4.57±0.13d | $3.88 \pm 0.14b$ | 97.5±5.80b |
| BHS ₃ | 3.04±0.03a | 32.4±0.70a | 5.87±0.13a | 63.4±0.70a | 9.44±0.90a | 6.23±0.94a | 132.1±7.03a |
| BHS_4 | 2.03±0.37d | $23.2{\pm}0.29b$ | 4.33±0.19b | 45.3±1.03b | 5.46±0.31c | 3.88±0.33b | 97.5±4.84b |
| BHS_5 | 1.86±0.10e | 21.7±0.38c | 4.11±0.22b | 42.6±0.70c | 6.26±0.06b | $3.98 \pm 0.10b$ | 92.4±3.82b |
| BHS_6 | 1.65±0.09f | 17.3±0.38e | 3.89±0.22b | 36.8±0.81d | 5.49±0.22c | 2.38±0.22c | 87.6±3.52b |
| LSD _{0.05} | 0.12 | 1.108 | 0.496 | 2.179 | 1.064 | 1.112 | 3.720 |
| <i>p</i> -value | < 0.0001** | < 0.0001** | < 0.0165* | < 0.0001** | < 0.0001** | < 0.0003** | < 0.0375* |

Mean values within columns followed by the same letter are; ** and * indicate respectively differences at $P \le 0.01$ and $P \le 0.05$ probability level, ^{NS} indicates not significant difference. Means followed by the same letter in each column are not significantly different according to the LSD test (P < 0.05). Total Chls; total chlorophyll content, FW; fresh weight, DW; dry weight, and No.; number.

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3.4. Effect of silymarin (Sm), iodine (I), and bee honey solution (BHS) on growth traits and pods yield of *Vicia faba* plants

The comparison among the best concentrations of Sm (Sm₁), iodine (I₃), and BHS (BHS₃) was depicted in **Table** 7. Foliar spray with BHS₃ generated best (significant) growth traits and pods

yield compared to Sm_1 and iodine (I₃). Foliar spraying with BHS₃ increased T.Chls content by 13.4 and 11.8%, SL by 20.0 and 17.4%, NLP by 20.0 and 22.8%, LAL by 15.7 and 13.4%, PFW by 19.9 and 6.19%, PDW by 36.3 and 4.71%, and pods yield by 20.0 and 22.7% compared to Sm_1 and I_3 , respectively.

Table 7. Effect of spraying with silymarin (Sm) on some growth traits and pods yield of *Vicia faba* plants

| Treatments | T.Chls (mg | Shoot | Leaves No. | Leaf area | FW | DW | Pods yield |
|---------------------|---------------------|-------------------|---------------------|--------------------|--------------------------|--------------------------|------------------------|
| | g ⁻¹ FW) | length (cm) | plant ⁻¹ | (cm ²) | (g plant ⁻¹) | (g plant ⁻¹) | (g pot ⁻¹) |
| Sm ₁ | 2.68±0.03b | 27.0±0.33b | 4.89±0.11b | 54.8±0.39b | 7.87±0.23c | 4.57±0.03c | 110.1±5.83b |
| I ₃ | $2.72{\pm}0.03b$ | $27.6 \pm 0.44 b$ | 4.78±0.11b | $55.9 \pm 0.29 b$ | $8.89{\pm}0.27b$ | $5.95{\pm}0.06b$ | $107.7 \pm 5.71b$ |
| BHS ₃ | $3.04{\pm}0.03a$ | 32.4±0.70a | 5.87±0.13a | 63.4±0.70a | 9.44±0.90a | $6.23{\pm}0.94a$ | 132.1±7.03a |
| LSD _{0.05} | 0.20 | 0.92 | 0.25 | 1.82 | 0.85 | 0.20 | 2.55 |
| <i>p</i> -value | 0.02* | 0.14* | 0.44* | 0.02* | 0.23** | 0.14** | 0.14** |

Mean values within columns followed by the same letter are; ** and * indicate respectively differences at $P \le 0.01$ and $P \le 0.05$ probability level, ^{NS} indicates not significant difference. Means followed by the same letter in each column are not significantly different according to the LSD test (P < 0.05). Total Chls; total chlorophyll content, FW; fresh weight, DW; dry weight, and No.; number.

plant growth biostimulants (PGBs), including silymarin (Sm), iodine (I), and diluted bee honey solution (BHS), support plant growth and productivity by regulating the metabolic processes through complex and various mechanisms linked to plant metabolic pathways (Rady et al., 2021; Ali et al., 2022; Hassan et al., 2023). In this study, foliar spraying of Vicia faba plants with the tested PGBs (e.g., Sm, I, and BHS) may stimulate several physio-biochemical processes, increase photosynthetic pigments (total chlorophyll content), plant growth traits, and yield (Rady et al., 2021; Ali et al., 2022; Hassan et al., 2023), especially when Sm, I, and BHS were applied at 0.200 mg, 24.9 mg, or 7.5 g L^{-1} , respectively.

As presented in **Table 3**, the analysis of bee honey showed that this promising tool for sustainable cultivation is a growth biostimulator plant for encouraging V. faba plant growth and production even under stress conditions (Rady et al., 2021; Hassan et al., **2023**). BHS is rich in osmoprotectants (e.g., proline, total amino acids, and soluble sugars), various soluble sugars fructose. glucose. sucrose. (e.g., maltose, etc.), and different nutrients (e.g., K, P, Mg, Ca, etc.). Additionally, it contains iodine with suitable content and high value of vitamin C and possesses high value of DPPH radicalscavenging activity (18.6 mM Trolox eq. L^{-1}), which is widely used to screen antioxidant activity to prevent lipid peroxidation in plant tissues (Semida

et al., 2019; Taha et al., 2020), which confers the antioxidant property of BHS.

Sm and iodine are known to have positive effects on stimulating several physio-biochemical processes, increasing photosynthetic pigments (total chlorophyll content), plant growth traits, and productivity (Ali et al., 2022; Hassan et al., 2023). However, in this study (Tables 4-7), the results obtained showed that BHS was superior to Sm and iodine in increasing total chlorophyll content, plant growth traits, and pod yield of V. faba plants. These positive results can be attributed to that BHS as a plant growth biostimulator contains several biostimulants that act simultaneously in network mechanisms. including growth-related metabolites of BHS such as proline, soluble sugars, amino acids. antioxidants, vitamins, and mineral nutrients, which support plants their growth to increase and development under normal or stress conditions (Alzahrani and Rady, 2019; Desoky et al., 2020; Rady et al., 2021; Hassan et al., 2023).

In the current study, leaf chlorophyll content was markedly increased by

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foliar-applied BHS. These results may be related to maintaining cell membrane integrity and increasing leaf BHS supplementation. RWC by Additionally, BHS is rich in nutrients to maintain intercellular hemostasis of required chlorophyll ions for biosynthesis, thus improving the efficiency of photosynthetic the machinery (Semida et al., 2019; Rady et al., 2021; Hassan et al., 2023) of V. faba plants. Nevertheless, foliar-applied BHS induced ion hemostasis and increased mineral nutrient contents of plants to increase root uptake surfaces resulting from increased root system volume, and/or increased accumulation of osmoprotectants to balance the osmotic pressure in organelles, thus mainlining cell turgor and improving nutritional status and water uptake (Semida et al., 2019; Rady et al., 2021; Hassan et al., 2023). The positive result regarding increased chlorophyll content in the leaves of V. faba plants was reflected positively in increasing the efficiency of photosynthesis process, and thus increasing the plant growth traits and the translocation of photosynthesized substances to increase pods yield (Tables 4-7).

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