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## Increasing Growth, Yield and Bioactive Compounds of Strawberry by Bio-Stimulants

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### ABSTRACT



Bio-stimulants have obtained global attention due to their beneficial impacts on the growth and development of several of crops. The current experiment was carried out during two winter seasons to assess the impact of different bio-stimulant substances on plant vigor, yield, and quality attributes in two strawberry cultivars (Fortuna and Festival) in a plastic greenhouse. Our treatments were: 1) humic acid 15% (10 ml L-1); 2) fulvic acid 15% (10 ml L-1); 3) potassium citrate (2 g L-1); 4) potassium silicate (2 g L-1); 5) salicylic acid (1 g L-1); 6) calcium carbonate (2 g L-1); and 7) control (water). Strawberry plants were treated weekly with each bio-stimulant, started after 15 days of transplanting and repeated 10 times. The most effective bio-stimulants were calcium carbonate and potassium silicate. Calcium carbonate enhanced all studied traits except dry matter, alkaloids concentration, antioxidant activity in leaves, and titratable acidity, while potassium silicate improved all traits except dry matter and alkaloids in leaves. Potassium citrate showed the highest value of flavonoids in leaves, firmness, and TSS. Humic acid exhibited the highest antioxidant activity in leaves and titratable acidity. Fulvic acid showed the highest total carbohydrates. The interaction between the cultivars and bio-stimulants was different in all traits. Significant differences were found among cultivars in all traits except total alkaloids, phenolics compounds, antioxidant activity in leaves, early yield, total yield, average fruit weight, TSS, and vitamin C in fruits (1st year) and number of leaves/plants, antioxidant activity, carbohydrates in leaves and total yield (2nd year).

Keywords: strawberry; bio-stimulants; productivity and quality.

#### INTRODUCTION

The strawberry plant (Fragaria × ananassa Duch.) is considered one of the most globally distributed crops (Chandler et al., 2012). In addition to being a natural source of nutrients and phytochemicals like folic acid, potassium, ascorbic acid, and fiber, strawberries also have health benefits that include preventing cancer, heart disease, blood sugar and pressure regulation, inflammation reduction, cholesterol reduction, and aging prevention (AbdusSamee et al., 2023). Egypt is one of the significant players in the world strawberry market, ranked as the fourth largest strawberry producing country after China, USA, and Turkey. In 2022 Egypt produced 637842.16 tons and exported 45210.15 tons with an export value of about 117540000 USD, making Egypt the sixth largest strawberry exporter worldwide. The Ministry of Agriculture in Egypt announced that the production of strawberry plants during 2019/2020 season was 433945 tons, cultivated in 26756 feddans, while average production was 17.08 tons / feddan.

Bio-stimulants have obtained global attention due to their beneficial impacts on the growth and development of several crops. Bio-stimulants involve the utilization or use of chemical substances, physical methods, or biological agents to induce a positive response to improve growth, development, and quality of several crops, and induce biotic and abiotic stress tolerance alongside increased nutrient uptake (Soppelsa et al., 2019; Garza-Alonso et al., 2022). Recently, many former studies documented the positive effects of the application of bio-stimulants on different crops including chemical and biochemical substances.

Humic substances are complex organic molecules, naturally developed in soil as a result of the long-term microbial degradation of plant and animal waste. Humic substances are categorized based on solubility characteristics of humin, fulvic, and humic acids (Ore et al., 2023). Zydlik and Zydlik (2023) reported that humic acid application was a suitable alternative for mineral fertilization, which led to maximized soil enzyme activity, an increase in leaf area by 60%, and a significant enhancement in flower number, fruit number, and fruit set, which positively affected on yield over mineral fertilizers. Ullah et al. (2017) evaluated the effect of three levels of humic acid (1.5, 3.0, and 4.5ml L<sup>-1</sup>), and found that using 3ml L<sup>-1</sup> produced more strawberry fruits and the highest value of total carotenoids. Chakraborty et al. (2023) studied the response of two cultivars of strawberry (Camarosa and Nabila) to humic acid and seaweed extract and found that foliar application of 2.0 ml L<sup>-1</sup> humic acid with 1.0 ml L<sup>-1</sup> seaweed extract significantly enhanced the plant growing, number of leaves, flowers and fruits, as well as, fruit weight, fruits length and yield than control, while the maximum total soluble solids (TSS %), vitamin c, total sugar were exhibited with 2.0 ml L<sup>-1</sup> humic acid + 2.0 ml L<sup>-1</sup> seaweed extract.

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Salicylic acid is a plant hormone and produced naturally in plants, and is considered a phenolic compound (Hassoon and Abduljabbar, 2020). Salicylic acid enhances plant vigor and fruit quality traits (Mohamed et al.,2017, Youssef et al., 2017) and improves the strawberry plant's tolerance for different abiotic stresses such as salinity stress (Roshdy et al., 2021), drought stress (Dakheel et al., 2022), and enhanced strawberry plant resistance for biotic stresses such as gray mold disease (Emam et al., 2023) and spider mite (De Resende et al., 2021).

Potassium citrate as a bio-stimulate enhanced strawberry plant's growth parameters such as plant length, number of crowns, runners, and leaves as well as, leaves fresh, and dry weight for leaves and enhanced the early, total, and marketable yield, especially with using mineral source for nitrogen over compost manure, while the Spraying of potassium citrate improved fruit weight, TSS%, and vitamin C with compost manure (Awad et al., 2010). Potassium citrate induced relative resistance to the two-spotted spider mite in strawberry plants (Abdelwines and Ahmed, 2022). Foliar application with potassium citrate improves the growth and productivity of several vegetable crops like potatoes (Mansour and El-Metwaly, 2019), hot peppers (Ghoname et al., 2009), tomatoes (Zakher and Elashry, 2016) and peas (El-Sayed et al., 2019).

Potassium silicate played an important role as a biostimulant in strawberry plants, achieved significant plant growth improvement, maximized fruit yield, and enhanced fruit quality when used at 0.6 g/L as a foliar application (Nada, 2020). EL-Sayed et al., (2023) found that utilizing potassium silicate on strawberry plants as foliar application significantly improved leaf number, chlorophyll, biomass, dry matter, and leaf area, in addition to fruit length, weight, and diameter, which led to a significant increment in both total and marketable yield. Hussain et al. (2023) reported that spraying strawberry plants three times with potassium Silicate decreases whitefly and aphid incidence by an increasing of the epidermal cuticle layer thickness and leaf thickness. Also, potassium silicate promoted different abiotic and biotic stress tolerances, like salinity (Yaghubi et al., 2019), water deficit (Dehghanipoodeh et al., 2018), and leaf blight disease resistance (Abd-El-Kareem et al., 2019).

A few former studies were found on the effect of foliar spray of calcium carbonate on plants. Foliar spray with calcium carbonate on gooseberry plants significantly increased fruit number/plant, weight, and size of fruit (Shukla et al., 2011). Calcium carbonate induced relative resistance to the two-spotted spider mites in strawberry plants (Abdelwines and Ahmed, 2022). Calcium carbonate was utilized as a biological control agent for powdery mildew in strawberry plants and found a reduction in disease incidence (Pertot et al., 2008). This experiment aimed at comparing the effects of different bio-stimulants i.e., humic acid, fulvic acid, salicylic acid, potassium citrate, potassium silicate, and calcium carbonate on plant vigor, yield, and quality attributes of strawberry.

#### **MATERIALS AND METHODS**

#### 1. Plant materials and the experimental layout.

Our experiment was performed during the two winter seasons of 2017/2018 and 2018/2019 to assess and compare the effects of different bio-stimulants on strawberry plant vigor, yield, and quality attributes in a plastic greenhouse of the vegetable crops department, Cairo University, Giza province, Egypt. The two strawberry cultivars (Fortuna and Festival) seedlings were transplanted in the first week of October in four rows/terraces with 25 cm between transplants and between rows on each terrace. Each experimental plot was about 6 m<sup>2</sup> (5 m length x 1.2 m width). Terraces were covered with black plastic mulch. Drip irrigation was applied with two drip lines/ terrace; also spry irrigation system was

used for 10 days only after transplanting to accelerate plant development. Common agricultural practices including diseases and pest management were applied according to the Ministry of Agriculture and Land Reclamation, Egypt.

#### 2. Treatments

The experimental treatments were: 1) humic acid 15% (10 ml L<sup>-1</sup>); 2) fulvic acid 15% (10 ml L<sup>-1</sup>); 3) potassium citrate (2 g L<sup>-1</sup>); 4) potassium silicate (2 g L<sup>-1</sup>); 5) salicylic acid (1 g L<sup>-1</sup>); 6) Calcium carbonate (2 g L<sup>-1</sup>); and 7) control (water). All treatments were added as foliar application except, humic acid was added manually as soil application. Strawberry plants were sprayed weekly, starting 15 days after the transplanting, and repeated 10 times. The experiment was performed using a randomized complete block design with 4 replicates in a split-plot design. Two strawberry cultivars as main plots, while bio-stimulants as split-plots.

#### 3. Data recorded

#### Vegetative traits and leaves chemical attributes

Agronomic traits were measured after three months of strawberry transplanting, except leaf area/plant measured after two months to measure the quick response. Five plants were randomly selected to assess the number of leaves/plants, leaf dry matter, and leaf area/ plant (Pandey and Singh, 2011). Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids) were assessed spectrophotometrically following the method of Mitic et al. (2013). The Folin-Ciocalteu method was used to determine phenolic concentration (Meda et al., 2005); meanwhile, the colorimetric method using aluminum chloride was used to measure flavonoids concentration (Chang et al., 2002). The phenol-sulfuric acid method was applied to assess the total carbohydrates (Dubois et al., 1956). Antioxidant activity was evaluated by the method of stable 2,2-diphenyl-1picrylhydrazyl radical (Kedare and Singh, 2011).

#### Yield and fruit quality attributes

Strawberry fruits were harvested at full mature stage from the whole plot for each treatment and the first 5 pickings were expressed as early yield (kg/m<sup>2</sup>). Total yield (kg/m<sup>2</sup>) was accumulatively determined from each plot for each treatment during the harvesting season. Average fruit weight was assessed as the mean weight of ten fruits. Fruit firmness was determined by a food pressure tester model FT011. Total soluble solids (TSS) were measured by a digital refractometer (model PR101, Co. Ltd., Tokyo, Japan). Titratable acidity was assessed in fresh strawberry fruits using 0.1 N NaOH solution with phenolphthalein as an indicator (AOAC, 1990). Ascorbic acid (Vitamin C) was assessed in ripe strawberry fruits using 2, 6 dichlorophenol indophenol dye by a titrimetric method (AOAC, 1990). Anthocyanin was measured in strawberry fruits according to Pirie and Mullins's method 1976.

#### 4. Statistical analysis

Data were statistically analyzed by MSTAT-C v. 2.1 software program (Michigan State University, Michigan, USA) using two-way ANOVA. The means of treatment were compared by Duncan's multiple range test at the 0.05 level of probability (Gomez and Gomez, 1984).

#### **RESULTS AND DISCUSSION**

Increasing demand for bio-stimulants due to their positive effects on plant growth parameters, especially in poor soils, and unsuitable conditions such as abiotic and biotic stresses, and reduce the amounts of required fertilizers for plants and improve their productivity and enhance their tolerance to different stresses (De Vasconcelos and Chaves, 2019).

# Effect of bio-stimulants, cultivars and their interaction on number of leaves/plants and leaf area.

The individual effect of stimulants on strawberry plants is shown in Figure 1. Potassium silicate demonstrated the highest number of leaves/plants in both seasons with no significant difference with salicylic acid and calcium carbonate in the second season (Figure 1A). Fulvic acid caused a significant reduction in the number of leaves/plants in both seasons. Potassium silicate recorded the highest value of leaf area in the first season compared to all other treatments followed by calcium carbonate (Figure 1B). Calcium carbonate and potassium citrate recorded the largest leaf area in the second season with no significant differences. The lowest value of leaf area per plant was recorded in fulvic acid treatment in the first season and control treatment in the second season.

Concerning the interaction between cultivars and biostimulants, Table 1 revealed that significant differences were found among bio-stimulants in the two cultivars for the number of leaves and leaf area per plant. Strawberry plants treated with potassium silicate displayed the highest number of leaves/ plants in the Fortuna cultivar, followed by calcium carbonate in Festival cultivar in the first season. In the second season, the highest significant no. of leaves/plants was recorded in salicylic acid and potassium silicate in the Furtuna cultivar and calcium carbonate in Festival cultivar without significant differences among them.

The plants of the Fortuna cultivar treated with potassium silicate exhibited the largest leaf area in the first season, followed by plants of Festival cultivar treated with calcium carbonate. while the treatment of fulvic acid, potassium citrate, and calcium carbonate recorded the largest leaf area in Festival cultivar in the second season compared to all other treatments without significant differences among them.

Cultivars exhibited a significant effect on the number of leaves/plants in the first year only, while Cultivars displayed a significant effect on leaf area in both years.

Potassium ( $K^+$ ) as a cation, and calcium ( $Ca^{2+}$ ) are the most prevalent inorganic compounds in plant cellular media (Sardans and Peñuelas, 2021). Many former studies confirmed potassium's role in numerous physiological processes such as control of cellular growth, controlling water balance, in addition to metabolite and nutrient transport (Sardans and Peñuelas, 2021). Potassium application led to improved plant growth characteristics like number of leaves, biomass of the plant, leaf area, and dry matter %. Growth improvement by K could be due to their role in physiological processes such as nitrogen usage, protein biosynthesis, and cell development (Coskun et al., 2017). EL-Sayed et al (2023) reported that improvement in strawberry growth parameters such as the number of leaves and leaf area by using three concentrations of potassium silicate as a foliar application. Nada (2020) reported an enhancement in the number of leaves and leaf area due to spraying strawberry plants with four concentrations of potassium silicate. Previous studies confirmed an increment in both leaf area and the number of leaves after foliar spraying with potassium silicate separately and in mixed treatment with acetyl-salicylic acid on tomato plants (El-Sayed et al., 2023). In contrast, no effects on leaf area and leaf number were shown in strawberry plants using potassium silicate under water stress (Dehghanipoodeh et al., 2018). Also, potassium citrate alone and combined with boron led to improved vegetative parameters in Sweet Charlie strawberry cultivar (Awad et al., 2010).

A vital role was played by calcium as an essential element required for plant development in normal and stressful conditions. It is important for membrane stability and cell wall and plays a second messenger in many physiological processes (Thor, 2019). Furthermore, the transport of carbohydrates that are produced during photosynthesis to sinks is accelerated by calcium (Navazio et al.,

2020). Former studies in agreement with our results, such as Rozbiany and Taha, (2020) who found that positive increment in all vegetative characters due to foliar application of calcium on strawberry plants. El-Sayed et al. (2023) reported that leaf area and number of leaves were enhanced as a result of calcium foliar application on strawberry plants. **Effect of bio-stimulants, cultivars and their interaction on leaves chemical attributes.** 

#### a- Photosynthetic pigments concentration:

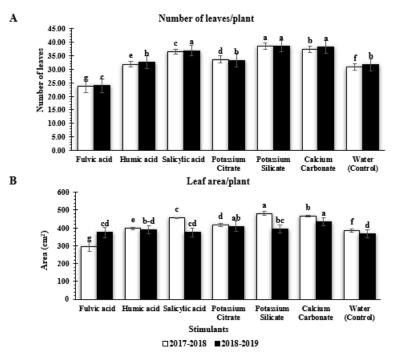
Data in Figure 2 showed the individual effects of biostimulants on strawberry plants for chlorophyll a, b, total chlorophyll, and total carotenoids. Calcium carbonate recorded the highest value of chlorophyll a content in both seasons, with no significant differences with salicylic acid and potassium silicate in the second season (Figure 2A). In contrast, fulvic acid showed a reduction in chlorophyll a over control plants in the first season. Calcium carbonate in both seasons, potassium citrate, and potassium silicate in the second season exhibited the highest content of chlorophyll b (Figure 2B). Regarding the total chlorophyll concentration, calcium carbonate in both seasons and potassium silicate in the second season gave the highest value compared to all other treatments (Figure 2C). Fulvic acid showed a reduction in total chlorophyll over control plants in the first season. Regarding total carotenoids, all bio-stimulants caused a significant reduction in total carotenoid concentration, except fulvic acid in the second season (Figure 2D).

Regarding the interaction between the cultivars and bio-stimulants, Table 2 displayed that significant differences were found among the bio-stimulants in the two cultivars for chlorophyll a, b, total chlorophyll and total carotenoids. Plants of the Festival cultivar treated with calcium carbonate exhibited the maximum value of chlorophyll a in both seasons. Foliar application of Fortuna plants with potassium citrate recorded the highest chlorophyll a concentration after calcium carbonate in first season, while foliar application of Festival plants with potassium silicate and potassium citrate in second season ranked second in chlorophyll a concentration. Concerning chlorophyll b concentration, in the first season spraying Fortuna plants treated with calcium carbonate recorded the highest content, and spraying Festival plants with calcium carbonate ranked the second. Meanwhile, the foliar application of both cultivars with potassium citrate, calcium carbonate, and potassium silicate on Festival showed the highest concentration of chlorophyll b in the second season. concerning total chlorophyll concentration, foliar application with calcium carbonate in both cultivars during the first season and in Festival cultivar in the second season, in addition to potassium silicate in Festival cultivar in the second season displayed the highest significant concentration

of total chlorophyll. Concerning total carotenoids, plants of control in both cultivars in first season and control plants in Fortuna, in addition to spraying Festival with fulvic acid showed the highest concentration of total carotenoids in second season, while other treatments caused a significant reduction in total carotenoids in both cultivars.

Cultivars demonstrated significant effects on chlorophyll a, b, total chlorophyll, and total carotenoids in both years.

Our results agree with former studies, which found that foliar spraying on two different cultivars of strawberry (Albion and Festival) with calcium significantly improved chlorophyll concentration (Rozbiany and Taha, 2020). Foliar spraying with potassium silicate under normal or water stress conditions on strawberry achieved a significant increment in chlorophyll index (Dehghanipoodeh et al., 2018). Also, foliar spraying with three concentrations (4, 6, and 8%) of potassium silicate and three concentrations of calcium significantly increased chlorophyll percentage in strawberry leaves (El-Sayed et al.,2023). Nada (2020) reported that using potassium silicate with three concentrations (0.2,0.4 and 0.6 g L<sup>-1</sup>) enhanced chlorophyll a, b, and total, in addition to total carotenoids in strawberry leaves. In potatoes, foliar spraying with potassium citrate and potassium silicate enhanced total chlorophyll content in leaf tissues (Mousa et al., 2023).



Columns with the same letter represent values that are not significantly different according to Duncan's multiple range test (p < 0.05). Vertical bars represent  $\pm$  standard error of the mean.

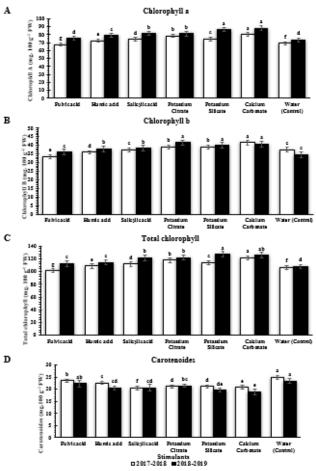
Fig. 1. Canopy parameters (A: number of leaves/plant and B: leaf area) of two strawberry cultivars treated with some stimulants in greenhouse conditions during the 2017-2018 and 2018-2019 winter seasons.

| Table 1. Canopy attributes (number of leaves/plant and leaf area) of strawberry cultivars Fortuna and Festival treated | l |
|--|---|
| with some bio-stimulants in greenhouse conditions during the 2017-2018 and 2018-2019 winter seasons.                   |   |

| Stimulants /       | Ň                | lo. of Le | aves/plant |     | Le           | Leaf area/ plant (cm <sup>2</sup> ) |              |     |  |  |  |  |
|--------------------|------------------|-----------|------------|-----|--------------|-------------------------------------|--------------|-----|--|--|--|--|
| Cultivars          | Fortuna          | L         | Festival   |     | Fortuna      |                                     | Festival     |     |  |  |  |  |
|                    |                  |           |            | 20  | 17-2018      |                                     |              |     |  |  |  |  |
| Fulvic acid        | 17.6±0.83        | h         | 29.76±1.26 | g   | 219.17±2.64  | 1                                   | 370.99±4.12  | j   |  |  |  |  |
| Humic acid         | 31.16±1.27       | f         | 32.51±2.09 | e   | 388.48±4.15  | i                                   | 404.31±7.55  | g   |  |  |  |  |
| Salicylic acid     | 36.85±1.27       | с         | 36.15±1.52 | cd  | 460.28±4.76  | с                                   | 450.65±5.03  | e   |  |  |  |  |
| Potassium Citrate  | 31.91±1.84       | ef        | 35.17±1.70 | d   | 396.55±5.75  | h                                   | 437.94±5.48  | f   |  |  |  |  |
| Potassium Silicate | 40.63±0.74       | а         | 36.27±1.73 | cd  | 508.70±2.82  | а                                   | 451.63±5.59  | de  |  |  |  |  |
| Calcium Carbonate  | 36.50±1.50       | с         | 38.30±2.01 | b   | 455.00±4.90  | d                                   | 476.94±7.00  | b   |  |  |  |  |
| Water (Control)    | 29.27±1.88       | g         | 32.41±1.45 | e   | 363.49±6.07  | k                                   | 403.76±4.69  | g   |  |  |  |  |
| Mean               | 31.98            | b         | 34.37      | а   | 398.81       | b                                   | 428.03       | a   |  |  |  |  |
|                    |                  |           |            | 20  | 18-2019      |                                     |              |     |  |  |  |  |
| Fulvic acid        | $18.43 \pm 1.68$ | g         | 29.51±2.58 | f   | 314.20±33.27 | g                                   | 436.40±18.33 | a-c |  |  |  |  |
| Humic acid         | 30.91±2.60       | ef        | 34.27±4.20 | b-e | 414.72±24.37 | cd                                  | 365.60±34.90 | ef  |  |  |  |  |
| Salicylic acid     | 37.85±2.59       | ab        | 35.83±3.11 | bc  | 376.70±41.79 | d-f                                 | 373.20±35.64 | ef  |  |  |  |  |
| Potassium Citrate  | 31.64±3.86       | d-f       | 34.87±3.52 | b-d | 359.70±40.68 | f                                   | 457.70±26.89 | ab  |  |  |  |  |
| Potassium Silicate | 41.20±1.52       | а         | 35.95±3.58 | b   | 370.30±35.12 | ef                                  | 418.90±28.94 | bc  |  |  |  |  |
| Calcium Carbonate  | 36.19±3.07       | b         | 40.10±4.02 | а   | 401.90±32.66 | c-e                                 | 469.5±23.72  | а   |  |  |  |  |
| Water (Control)    | 31.13±7.60       | d-f       | 32.15±3.01 | c-f | 353.20±33.32 | fg                                  | 378.50±32.57 | d-f |  |  |  |  |
| Mean               | 32.47            | а         | 34.66      | а   | 370.09       | b                                   | 414.25       | а   |  |  |  |  |

'Mean values followed by a letter in common were not significantly different according to Duncan's multiple range test (p < 0.05). Mean value ± standard error:

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Columns with the same letter represent values that are not significantly different according to Duncan's multiple range test (p < 0.05). Vertical bars represent  $\pm$  standard error of the mean.

- Fig. 2. Leaf pigments content (chlorophyll a (A), chlorophyll b (B), and total chlorophyll (C); and carotenoids (D)) of two strawberry cultivars treated with some stimulants in greenhouse conditions during the 2017-2018 and 2018-2019 winter seasons.
- Table 2. Leaf photosynthetic pigments concentration (chlorophylls a, b, and total chlorophyll; and carotenoids) of strawberry cultivars Fortuna and Festival treated with some stimulants in greenhouse conditions during the 2017-2018 and 2018-2019 winter seasons.

| 201/                           | 2017-2018 and 2018-2019 winter seasons. |       |                                   |        |                 |      |                                   |        |                 |   |                   |        |              |     |                                    |       |
|--------------------------------|---|-------|-----------------------------------|--------|-----------------|------|-----------------------------------|--------|-----------------|---|-------------------|--------|--------------|-----|------------------------------------|-------|
| Stimulants /                   |   |       | ophyll A<br>0 g <sup>-1</sup> FW) |        |                 |      | ophyll B<br>0 g <sup>-1</sup> FW) |        |                 | Total chlorophyll<br>(mg. 100 g <sup>-1</sup> FW) |                   |        |              |     | rotenoids<br>0 g <sup>-1</sup> FW) |       |
| Cultivars                      | Fortuna Festival                        |       |                                   | Fortun | Festival        | l    | Fortuna                           |        | U /             | Festival  |                   |        | Festiva      | al  |                                    |       |
|                                |   |       |                                   |        |                 |      |                                   | 20     | 017-2018        |   |                   |        |              |     |                                    |       |
| Fulvic acid                    | 7029±2.96                               | h     | 65.12±2.74                        | j      | 34.76±1.46      | i    | 32.21±1.36                        | j      | 105.05±4.42     | j   | 97.32±4.10        | k      | 23.67±1.00   | с   | 2332±098                           | d     |
| Humic acid                     | 73.93±3.11                              | e     | 7131±3.00                         | g      | 3657±154        | g    | 35.26±1.48                        | h      | 110.50±4.65     | f   | 106.58±4.49       | i      | 22.91±0.96   | e   | 2231±094                           | f     |
| Salicylic acid                 | 7632±321                                | d     | 7240±3.05                         | f      | 37.74±1.59      | f    | 36.81±1.55                        | g      | 114.10±4.80     | e   | 109.22±4.60       | g      | 21.97±0.93   | g   | 1920±0.81                          | j     |
| Potassium Citrate              | 80.54±3.39                              | b     | 7691±324                          | d      | 39.83±1.68      | с    | 38.28±1.61                        | e      | 120.40±5.07     | b   | 115.20±4.85       | d      | 20.61±0.87   | i   | 21.73±091                          | h     |
| Potassium Silicate             | 70.67±2.98                              | h     | 79.01±3.33                        | с      | 39.01±1.64      | d    | 39.08±1.65                        | d      | 109.70±4.62     | fg  | 118.10±4.97       | с      | 21.64±0.91   | h   | 20.68±0.87                         | i     |
| Calcium Carbonate              | 78.73±332                               | с     | 81.73±3.44                        | а      | 42.77±1.80      | а    | 40.42±1.70                        | b      | 121.50±5.12     | а   | 122.10±5.14       | а      | 22.46±0.95   | f   | 1920±0.81                          | j     |
| Water (Control)                | 70.67±2.98                              | h     | 68.08±2.87                        | i      | 3494±1.47       | hi   | 39.78±1.68                        | с      | 105.60±4.45     | j   | 107.90±4.54       | h      | 25.37±1.07   | а   | 2445±1.03                          | b     |
| Mean                           | 74.45                                   | а     | 73.51                             | b      | 37.94           | а    | 37.4                              | b      | 11239           | а   | 11091             | b      | 22.66        | а   | 2155                               | b     |
|                                |   |       |                                   |        |                 |      |                                   | 20     | )18-2019        |   |                   |        |              |     |                                    |       |
| Fulvic acid                    | 73.64±4.13                              | h     | 7693±351                          | g      | 33.852.77       | f    | 38.22±1.67                        | cd     | 109.80±6.80     | f-h   | 115.10±5.17       | d-f    | 19.86±1.74   | fg  | 24.67±1.08                         | а     |
| Humic acid                     | 79.68±4.49                              | e-g   | ; 7826±333                        | fg     | 37.53±2.27      | сe   | 38.15±2.31                        | cd     | 113.50±6.48     | e-g   | 115.10±5.54       | d-f    | 21.26±1.29   | d-f | 19.82±1.25                         | fg    |
| Salicylic acid                 | 80.65±3.86                              | ef    | 82.19±328                         | de     | 36.64±3.00      | de   | 39.67±1.73                        | bc     | 119.40±6.75     | b-d   | 123.70±5.11       | bc     | 18.26±1.60   | gh  | 23.02±1.00                         | а-c   |
| Potassium Citrate              | 76.76±4.12                              | gh    | 86.19±325                         | bc     | 41.59±2.23      | ab   | 41.72±1.89                        | ab     | 118.40±6.28     | c-e   | 125.10±5.00       | b      | 20.83±1.12   | ef  | 22.03±1.01                         | c-e   |
| Potassium Silicate             | 84.67±4.28                              | cd    | 8793±3.05                         | b      | 37.92±2.18      | cd   | 42.08±2.29                        | а      | 122.60±6.38     | bc  | 131.70±5.39       | а      | 18.49±1.06   | gh  | 20.67±1.12                         | ef    |
| Calcium Carbonate              | 82.79±4.00                              | de    | 93.81±3.05                        | а      | 40.63±2.18      | ab   | 40.70±3.40                        | ab     | 120.00±5.96     | b-d   | 130.90±6.24       | а      | 20.97±1.12   | ef  | 1692±1.52                          | g     |
| Water (Control)                | 7026±2.80                               | i     | 7653±355                          | gh     | 35.30±1.48      | ef   | 33.99±3.33                        | f      | 107.10±4.37     | h   | 108.60±6.19       | gh     | 24.07±1.00   | ab  | 22.74±223                          | bd    |
| Mean                           | 78.34                                   | b     | 83.11                             | а      | 37.63           | b    | 3922                              | а      | 115.82          | b   | 121.44            | а      | 20.53        | b   | 21.41                              | а     |
| <sup>z</sup> Mean values follo | wed by a let                            | ter i | in common                         | were   | e not significa | ntly | different acco                    | ording | g to Duncan's i | multip  | ole range test (j | p <0.0 | 5). Mean val | ue∃ | standard e                         | rror. |

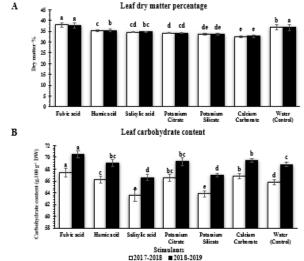
#### **b-** Dry matter and carbohydrates in leaves

Results in Figure 3 revealed the individual effects of bio-stimulants on strawberry plants for dry matter and

carbohydrates. Fulvic acid only showed a significant increment in dry matter % in the first season. In the second season, fulvic acid and the control treatment showed the

highest dry matter % while other treatments reduced dry mater % in strawberry leaves (Figure 3A). Regarding carbohydrates concentration, fulvic acid in both seasons showed the highest value followed by potassium citrate and calcium carbonate in both seasons with no significant difference between them, in addition to humic acid in the second season.

Regarding the interaction between cultivars and biostimulants, Table 3 demonstrated that significant differences were found among the effects of the bio-stimulants in the two cultivars for dry matter and carbohydrates. All bio-stimulants led to a significant reduction in dry matter % in both cultivars, except fulvic acid in both cultivars without a significant difference between fulvic acid treatment and control plants in the Fortuna cultivar only. The spraying Fortuna plants with fulvic acid in both seasons and Festival plants with potassium citrate in the second season displayed the highest carbohydrate concentration. Spraying Fortuna plants with humic acid in both seasons and Festival plants with potassium citrate in first the season ranked second in carbohydrate content.



Columns with the same letter represent values that are not significantly different according to Duncan's multiple range test (p < 0.05). Vertical bars represent  $\pm$  standard error of the mean.

Fig. 3. Leaf dry matter %and carbohydrate concentration of strawberry cultivars Fortuna and Festival treated with some stimulants in greenhouse conditions during the 2017-2018 and 2018-2019 winter seasons.

| Table 3. Leaf dry matter % and carbohydrate concentration of strawberry cultivars Fortuna and Festival treated with |
|---|
| some bio-stimulants in greenhouse conditions during the 2017-2018 and 2018-2019 winter seasons.                     |

| Stimulants /       | ]          | Dry matte | r (%)      | Total Carbohydrates (g.100g <sup>-1</sup> DW) |                  |    |            |     |  |  |  |
|--------------------|------------|-----------|------------|---|------------------|----|------------|-----|--|--|--|
| Cultivars          | Fortuna    |           | Festival   |   | Fortuna          |    | Festival   |     |  |  |  |
|                    |            |           |            | 2017-20                                       | 018              |    |            |     |  |  |  |
| Fulvic acid        | 39.72±0.38 | а         | 36.14±1.59 | bc  | 68.72±0.91       | а  | 66.16±0.75 | ef  |  |  |  |
| Humic acid         | 36.29±0.73 | b         | 34.41±0.14 | de  | 67.39±0.74       | bc | 65.01±0.62 | i   |  |  |  |
| Salicylic acid     | 34.81±0.39 | b-d       | 34.42±0.11 | de  | 61.58±1.02       | 1  | 65.56±1.06 | g-i |  |  |  |
| Potassium Citrate  | 34.71±0.35 | cd        | 33.71±0.17 | d-f   | 65.31±0.53       | hi | 67.67±0.79 | b   |  |  |  |
| Potassium Silicate | 34.63±0.32 | d         | 32.53±0.15 | fg  | 63.50±0.77       | k  | 64.17±0.73 | i   |  |  |  |
| Calcium Carbonate  | 33.12±0.19 | e-g       | 32.12±0.80 | g   | 66.57±0.70       | de | 67.07±0.56 | cd  |  |  |  |
| Water (Control)    | 40.04±0.25 | a         | 33.46±0.17 | d-g   | 65.59±0.69       | gh | 65.95±0.75 | fg  |  |  |  |
| Mean               | 36.18      | а         | 33.82      | b   | 65.52            | b  | 65.94      | a   |  |  |  |
|                    |            |           |            | 2018-20                                       | 019              |    |            |     |  |  |  |
| Fulvic acid        | 40.07±0.18 | а         | 36.04±1.60 | bc  | 71.60±0.70       | а  | 69.31±0.51 | cd  |  |  |  |
| Humic acid         | 36.75±0.61 | b         | 34.32±0.11 | de  | $70.48 \pm 0.50$ | b  | 67.60±0.42 | e   |  |  |  |
| Salicylic acid     | 35.07±0.27 | cd        | 34.35±0.09 | de  | 65.85±0.69       | f  | 67.33±0.60 | e   |  |  |  |
| Potassium Citrate  | 34.48±0.27 | de        | 33.82±0.12 | de  | 67.52±0.36       | e  | 70.99±0.54 | ab  |  |  |  |
| Potassium Silicate | 34.42±0.24 | de        | 32.44±0.11 | fg  | 66.73±0.52       | e  | 67.21±0.49 | e   |  |  |  |
| Calcium Carbonate  | 33.25±0.14 | ef        | 31.59±0.61 | g   | 69.50±0.47       | с  | 69.42±0.38 | с   |  |  |  |
| Water (Control)    | 40.21±0.19 | а         | 33.35±0.14 | ef  | 68.48±0.47       | d  | 69.08±0.51 | cd  |  |  |  |
| Mean               | 36.32      | а         | 33.70      | b   | 68.59            | а  | 68.70      | a   |  |  |  |

<sup>4</sup>Mean values followed by a letter in common were not significantly different according to Duncan's multiple range test (p < 0.05). Mean value ± standard error. Cultivars exhibited a significant effect on dry matter in both years, but carbohydrates in the first season only. Cultivars exhibited a significant effect on dry matter of the first season only.

Chakraborty et al., (2023) tested two concentrations of humic acid (1.0 and 2.0-ml L<sup>-1</sup>) on strawberry plants and found that both concentrations increased the total sugar concentration. Regarding potassium citrate, foliar application with a combination of potassium citrate 1% and boron 50 ppm on strawberry plants improved carbohydrate concentration under both mineral nitrogen and compost manure application as organic fertilizer (Awad et al., 2010). EL-Sayed et al (2023)

Our results agree with those of Abou El Hassan and Husein (2016) and Suh et al., (2014) who found that foliar spray with fulvic acid increased plant dry weight in tomato plants. Aminifard et al., (2012) reported positive enhancement in total carbohydrate concentration in pepper due to fulvic acid treatment. Concerning humic acid, reported a significant increase in total sugar due to foliar spraying with calcium and potassium silicate separately in strawberry plants.

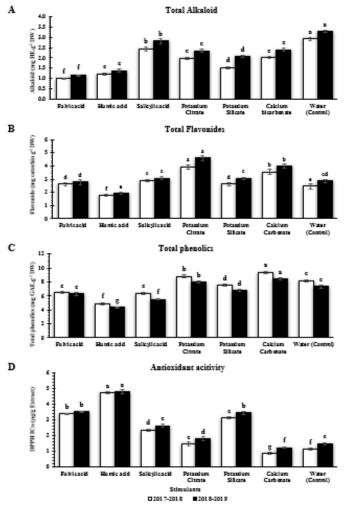
#### c- Non-enzymatic components

Data in Figure 4 displayed the individual effects of bio-stimulants on strawberry plants for non-enzymatic components, which include alkaloids, flavonoids, total phenolics, and antioxidant activity. All bio-stimulants showed negative effects on alkaloid concentration in leaves in both seasons (Figure 4A). Potassium citrate exhibited the highest significant flavonoid content followed by calcium carbonate in both seasons compared to other treatments (Figure 4B).

All bio-stimulants impaired total phenolics, except calcium carbonate and potassium citrate. Calcium carbonate showed the highest value of total phenolics, followed by potassium citrate in both seasons (Figure 4C). All biostimulants enhanced the antioxidant activity, except calcium carbonate, which caused a significant reduction. Humic acid recorded the highest significant antioxidant activity, followed by fulvic acid (Figure 4D).

Concerning the interaction between cultivars and biostimulants, Table 4 showed that significant differences were found among the effects of the bio-stimulants in the two cultivars for non-enzymatic components. All bio-stimulants reduced alkaloids in leaves over control plants in both cultivars, which showed the highest significant value of alkaloids. Regarding flavonoids, foliar application of Festival plants with potassium citrate in both seasons, calcium carbonate of Festival plants in the first season displayed the highest flavonoids content, meanwhile spraying Fortuna plants with potassium citrate in both seasons and calcium carbonate of Festival plants in the second season ranked second in flavonoids concentration.

Concerning total phenolics, all bio-stimulants had a negative effect on total phenolic concentration, except calcium carbonate and potassium citrate. Spraying the Fortuna cultivar with calcium carbonate recorded the maximum value, while spraying the festival cultivar with calcium carbonate ranked second in both seasons, in addition to potassium citrate in festival cultivar in the first season and in Fortuna in the second season. Concerning antioxidant activity, all bio-stimulants enhanced antioxidant activity, except potassium citrate and calcium carbonate which are equal to control treatments in both cultivars throughout both seasons. Humic acid foliar application on Fortuna cultivar demonstrated the highest antioxidant activity in both seasons, meanwhile, humic acid application in festival cultivar ranked second in both seasons.



Columns with the same letter represent values that are not significantly different according to Duncan's multiple range test (p <0.05). Vertical bars represent ± standard error of the mean.

Fig. 4. Non-enzymatic antioxidant leaf concentration (A: total alkaloids; B: total flavonoids; C: total phenolics; and D: antioxidant activity) of two strawberry cultivars treated with some bio-stimulants in greenhouse conditions during the 2017-2018 and 2018-2019 winter seasons.

| Table 4. Leaf concentration of non-enzymatic components (alkaloids, flavonoids, total phenolics, and antioxidant |
|--|
| activity) of strawberry cultivars Fortuna and Festival treated with some bio-stimulants in greenhouse            |
| conditions during the 2017-2018 and 2018-2019 winter seasons.  |

| Stimulants /                | Tota                       | lkaloids | Tota            | vonoids | Tota            | henolics   | Antioxidant activity    |         |                 |                       |                          |         |                 |    |               |    |
|-----------------------------|----------------------------|----------|-----------------|---------|-----------------|------------|-------------------------|---------|-----------------|-----------------------|--------------------------|---------|-----------------|----|---------------|----|
| Stimulants /<br>Cultivars - | (mg HE.g <sup>-1</sup> DW) |          |                 |         | (mg Ca          | tecł       | nin.g <sup>-1</sup> DW) |         | (mg G           | E.g <sup>-1</sup> DW) | DPPH IC50 (µg/g Extract) |         |                 | t) |               |    |
| Culuvars                    | Fortuna                    |          | Festival        |         | Fortuna         | a Festival |                         | Fortuna |                 | Festival              |                          | Fortuna | Festival        |    |               |    |
|                             |                            |          |                 |         |                 |            | 2                       | 017-    | 2018            |                       |                          |         |                 |    |               |    |
| Fulvic acid                 | $1.00\pm0.03$              | e        | $0.99 \pm 0.03$ | e       | $2.41 \pm 0.07$ | f          | $2.81\pm0.11$           | de      | $6.52 \pm 0.19$ | f                     | $6.47 \pm 0.16$          | fg      | $3.35 \pm 0.03$ | с  | $3.39\pm0.04$ | c  |
| Humic acid                  | $1.21\pm0.08$              | e        | $1.23\pm0.05$   | e       | $1.70\pm0.05$   | h          | $1.84\pm0.06$           | gh      | $4.64 \pm 0.07$ | i                     | $5.09\pm0.15$            | h       | 4.85±0.03       | а  | 4.63±0.05     | b  |
| Salicylic acid              | $2.44\pm0.17$              | b        | $2.42\pm0.15$   | b       | $3.00{\pm}0.07$ | с          | $2.80\pm0.08$           | de      | $6.17 \pm 0.15$ | g                     | $6.65 \pm 0.22$          | f       | $2.40\pm0.09$   | e  | 2.26±0.10     | e  |
| Potassium Citrate           | $2.01\pm0.05$              | с        | $1.93\pm0.11$   | с       | $3.76\pm0.16$   | b          | 4.13±0.26               | а       | $8.48 \pm 0.05$ | c                     | 9.13±0.29                | b       | $1.84\pm0.06$   | f  | $1.05\pm0.11$ | g  |
| Potassium Silicate          | $1.50\pm0.18$              | d        | 1.54±0.19       | d       | 2.51±0.21       | f          | $2.72\pm0.08$           | e       | $7.88 \pm 0.20$ | d                     | 7.15±0.10                | e       | $2.99 \pm 0.08$ | d  | 3.28±0.12     | с  |
| CalciumCarbonate            | $1.99\pm0.08$              | с        | $2.03\pm0.09$   | с       | $3.01 \pm 0.14$ | с          | $4.06 \pm 0.09$         | а       | 9.76±0.19       | а                     | $8.98 \pm 0.20$          | b       | $0.90{\pm}0.09$ | h  | $0.79\pm0.10$ | h  |
| Water (Control)             | $2.87\pm0.10$              | а        | $2.95\pm0.08$   | а       | $1.96\pm0.16$   | g          | $2.96 \pm 0.06$         | cd      | $7.87 \pm 0.20$ | d                     | $8.50\pm0.14$            | с       | $1.07\pm0.11$   | g  | 1.13±0.09     | g  |
| Mean                        | 1.860                      | a        | 1.867           | а       | 2.62            | b          | 3.04                    | а       | 7.33            | а                     | 7.42                     | а       | 2.48            | а  | 2.36          | а  |
|                             |                            |          |                 |         |                 |            | 2                       | 018-    | 2019            |                       |                          |         |                 |    |               |    |
| Fulvic acid                 | $1.16\pm0.02$              | g        | $1.16\pm0.03$   | g       | 2.33±0.25       | ef         | 3.23±0.05               | cd      | $6.94 \pm 0.18$ | d                     | $5.68\pm0.15$            | e       | 3.49±0.02       | cd | $3.55\pm0.03$ | с  |
| Humic acid                  | $1.44\pm0.02$              | f        | $1.31\pm0.11$   | fg      | $1.78\pm0.11$   | g          | $2.08\pm0.03$           | fg      | $4.41\pm0.11$   | f                     | 4.38±0.13                | f       | $4.99 \pm 0.02$ | а  | 4.61±0.20     | b  |
| Salicylic acid              | $3.02 \pm 0.06$            | b        | $2.60\pm0.20$   | с       | $3.02\pm0.22$   | d          | $3.11 \pm 0.04$         | d       | $5.46\pm0.13$   | e                     | $5.59{\pm}0.20$          | e       | $2.78 \pm 0.06$ | e  | 2.44±0.15     | f  |
| Potassium Citrate           | 2.30±0.04                  | d        | 2.37±0.15       | d       | 4.12±0.18       | b          | 5.09±0.12               | а       | 8.23±0.05       | b                     | 7.74±0.26                | с       | $2.09\pm0.04$   | g  | $1.49\pm0.18$ | h  |
| Potassium Silicate          | $2.06\pm0.06$              | e        | $2.07 \pm 0.06$ | e       | $3.05\pm0.16$   | d          | $3.03{\pm}0.04$         | d       | $6.90{\pm}0.18$ | d                     | $6.68\pm0.09$            | d       | $3.34 \pm 0.06$ | d  | 3.56±0.19     | с  |
| CalciumCarbonate            | $2.40\pm0.06$              | d        | $2.38\pm0.14$   | d       | $3.54 \pm 0.07$ | с          | $4.38 \pm 0.04$         | b       | $8.86 \pm 0.17$ | а                     | $8.01\pm0.18$            | bc      | $1.27\pm0.06$   | ij | $1.15\pm0.08$ | j  |
| Water (Control)             | $3.32 \pm 0.07$            | а        | $3.25\pm0.10$   | а       | $2.57 \pm 0.08$ | e          | $3.18 \pm 0.03$         | d       | $6.90{\pm}0.18$ | d                     | 7.81±0.13                | с       | $1.51\pm0.07$   | ĥ  | 1.43±0.15     | ĥi |
| Mean                        | 2.242                      | а        | 2.161           | а       | 2.91            | b          | 3.44                    | а       | 6.81            | а                     | 6.55                     | b       | 2.78            | а  | 2.60          | а  |

<sup>2</sup>Mean values followed by a letter in common were not significantly different according to Duncan's multiple range test (p <0.05). Mean value ± standard error.

Cultivars displayed a significant effect on flavonoids in both seasons and on total phenolics in the second season only, but they had a non-significant effect on alkaloids and antioxidant activity in both seasons.

Non-enzymatic components are produced by plants to defend against different stresses. In former studies, El-Sayed et al., (2023) revealed that foliar spraying with salicylic acid reduced the non-enzymatic components in tomato leaves such as total sugars and total phenolics. Aminifard et al., (2012) found that fulvic acid treatment increased total phenol and antioxidant activity while having no effects on total flavonoids in pepper plants. El-Sayed et al., (2023) found that calcium and potassium silicate didn't affect the total phenol concentration, while increased antioxidant capacity in strawberry plants. Aghaeifard et al., (2016) found a positive effect of salicylic acid on strawberry plants, that increased antioxidant capacity, while humic acid didn't affect this trait. **Effect of bio-stimulants, cultivars, and their interaction on yield and its quality** 

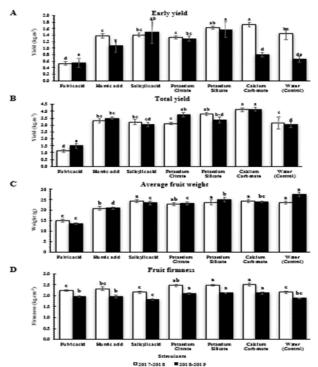
Results in Figure 5 revealed the individual effects of bio-stimulants which displayed significant differences between bio-stimulants for early yield, total yield, average fruit weight, and fruit firmness. Potassium silicate in both seasons showed the highest significant early yield with no significant differences with calcium carbonate in the first season, and salicylic acid in the second season (Figure 5A). Fulvic acid showed the lowest early yield in the first season compared to all other treatments and was equal to the control treatment in the second season. Calcium carbonate in both seasons, potassium silicate in the first season, and potassium citrate in the second season recorded the highest total yield, while fulvic acid produced the lowest total yield in both seasons (Figure 5B). Fulvic and humic acid in the first season and all bio-stimulants in the second season caused a significant reduction in average fruit weight (Figure 5C). Regarding firmness, potassium citrate, potassium silicate, and calcium carbonate showed the highest values in both seasons with no significant difference (Figure 5D).

Regarding the interaction between cultivars and biostimulants, Table 5 showed significant differences were found among the bio-stimulants in the two cultivars for early yield, total yield, average fruit weight and fruit firmness. Concerning the early yield, spraying the festival cultivar with potassium silicate in both seasons, calcium carbonate in the first season, and salicylic acid in the second season showed the highest early yield. Humic acid and potassium citrate ranked second in this trait in festival cultivar in the second season. Fulvic acid caused a significant reduction in early yield in both cultivars in the first year, while other biostimulants did not differ from plants of control.

Regarding total yield, calcium carbonate in both cultivars during the two seasons, potassium silicate in both cultivars in the first season, and humic acid in festival cultivar produced the highest total yield. Fulvic acid reduced total yield in both cultivars in both seasons. Other treatments did not differ from plants of the control.

Concerning the average fruit weight, fulvic acid in both cultivars and humic acid in Fortuna caused a significant reduction in this trait, while other treatments did not differ from plants of control in both cultivars in the first season. Fulvic acid and humic acid in both cultivars, in addition to salicylic acid and potassium citrate in the Fortuna caused a significant reduction in average fruit weight in the second season, while other treatments did not differ from plants of the control in both seasons. Concerning fruit firmness, all treatments except fulvic acid and salicylic acid in both cultivars, in addition to humic acid in Festival cultivar improved fruit firmness in the first season. Also, all treatments, except salicylic acid in both cultivars, in addition to humic and fulvic acid in Festival cultivar enhanced fruit firmness in the second season.

Cultivars displayed a significant effect on fruit firmness in both seasons, on early yield and average fruit weight in the second season only, but they had a nonsignificant effect on total yield in both years.



Columns with the same letter represent values that are not significantly different according to Duncan's multiple range test (p <0.05). Vertical bars represent ± standard error of the mean.

| Fig. 5. Yield component (A: early yield and B: total yields kg/m <sup>2</sup> ) and physical fruit quality parameters (C: average fruit |
|---|
| weight and D: fruit firmness) of two strawberry cultivars treated with some bio-stimulants in greenhouse                                |
| conditions during the 2017-2018 and 2018-2019 winter seasons.   |

Table 5. Yield component and physical fruit quality parameters of strawberry cultivars Fortuna and Festival treated with some bio-stimulants in greenhouse conditions during the 2017-2018 and 2018-2019 winter seasons.

| Cultivers (C) Early yield       |                   |      | ſ             | Total  | yield            |                      | Avera         | ge f   | ruit weight      | Fruit firmness |                  |                       |                  |       |                 |     |
|---------------------------------|-------------------|------|---------------|--------|------------------|----------------------|---------------|--------|------------------|----------------|------------------|-----------------------|------------------|-------|-----------------|-----|
| Cultivars (C)                   |                   | (kg  | g/m²)         |        |                  | (kg/m <sup>2</sup> ) |               |        |                  |                | (g)              | (kg/cm <sup>2</sup> ) |                  |       |                 |     |
| Stimulants (S)                  | Fortuna Festival  |      |               | Fortun | Fortuna Festival |                      |               |        | Fortuna Festival |                |                  |                       | Fortuna Festival |       |                 |     |
|                                 |                   |      |               |        |                  |                      |               | 2017   | -2018            |                |                  |                       |                  |       |                 |     |
| Fulvic acid                     | $0.39 {\pm} 0.06$ | e    | $0.67\pm0.02$ | e      | $0.91{\pm}0.13$  | d                    | 1.32±0.04     | d      | $14.64 \pm 0.60$ | e              | 15.45±1.02       | e                     | $2.35\pm0.02$    | b-e   | $2.14\pm0.04$   | e   |
| Humic acid                      | 1.34±0.14         | b-d  | 1.42±0.05     | a-d    | $3.25 \pm 0.33$  | bc                   | 3.36±0.13     | ab     | $18.92 \pm 0.48$ | d              | 23.03±0.73       | a-c                   | $2.43{\pm}0.06$  | a-c   | $2.22\pm0.13$   | c-e |
| Salicylic acid                  | 1.44±0.12         | a-d  | 1.39±0.07     | a-d    | $3.25 \pm 0.35$  | bc                   | 3.19±0.15     | bc     | $22.74{\pm}1.07$ | bc             | 25.86±0.28       | а                     | $2.19{\pm}0.06$  | de    | $2.16\pm0.09$   | e   |
| Potassium Citrate               | 1.37±0.09         | b-d  | 1.29±0.05     | cd     | $3.18 \pm 0.20$  | bc                   | 3.07±0.09     | bc     | $21.30\pm0.43$   | cd             | 24.48±0.53       | ab                    | $2.53\pm0.06$    | ab    | $2.41\pm0.11$   | a-d |
| Potassium Silicate              | $1.67\pm0.08$     | ab   | 1.59±0.05     | a-c    | $3.84\pm0.19$    | ab                   | 3.71±0.14     | ab     | $24.88 \pm 2.14$ | ab             | 22.66±1.57       | bc                    | $2.45\pm0.03$    | a-c   | $2.54{\pm}0.07$ | ab  |
| Calcium Carbonate               | 1.73±0.05         | а    | 1.72±0.13     | а      | $4.14\pm0.10$    | а                    | 4.12±0.28     | а      | $24.69{\pm}0.88$ | ab             | $24.02 \pm 1.15$ | a-c                   | $2.62\pm0.13$    | а     | $2.40\pm0.06$   | a-d |
| Water (Control)                 | 1.66±0.32         | ab   | 1.22±0.07     | d      | $3.82 \pm 0.75$  | ab                   | 2.45±0.15     | с      | $23.54{\pm}1.78$ | a-c            | 23.75±0.23       | a-c                   | $2.16\pm0.08$    | e     | $2.15\pm0.06$   | e   |
| Mean                            | 1.37              | а    | 1.32          | а      | 3.19             | а                    | 3.02          | а      | 21.53            | а              | 22.74            | а                     | 2.38             | а     | 2.28            | b   |
|                                 |                   |      |               |        |                  |                      | 4             | 2018   | 3-2019           |                |                  |                       |                  |       |                 |     |
| Fulvic acid                     | $0.28{\pm}0.05$   | f    | $0.83\pm0.17$ | de     | $1.13\pm0.08$    | g                    | 1.86±0.27     | f      | 13.24±0.09       | f              | 14.25±0.74       | f                     | $2.08\pm0.02$    | ab    | $1.86\pm0.03$   | d   |
| Humic acid                      | $0.58 \pm 0.05$   | ef   | $1.56\pm0.07$ | b      | $3.69\pm0.01$    | a-c                  | 3.27±0.01     | c-e    | 19.52±0.01       | e              | 22.57±0.44       | d                     | 2.10±0.01        | ab    | $1.84\pm0.05$   | d   |
| Salicylic acid                  | $0.62\pm0.19$     | e    | 2.39±0.19     | а      | $2.82\pm0.28$    | e                    | 3.28±0.14     | c-e    | $21.64\pm0.49$   | d              | 25.43±0.09       | b                     | $1.84\pm0.04$    | d     | $1.81\pm0.06$   | d   |
| Potassium Citrate               | 1.25±0.06         | bc   | 1.33±0.17     | b      | 3.81±0.26        | a-c                  | 3.73±0.23     | a-c    | $21.89\pm0.14$   | d              | 24.79±0.53       | bc                    | 2.20±0.01        | а     | $2.01{\pm}0.06$ | bc  |
| Potassium Silicate              | $0.99 \pm 0.07$   | cd   | 2.16±0.16     | а      | $3.47\pm0.41$    | b-d                  | 3.29±0.19     | c-e    | $25.33{\pm}1.96$ | b              | 24.96±0.74       | bc                    | 2.11±0.01        | ab    | $2.14\pm0.03$   | а   |
| Calcium Carbonate               | $0.78\pm0.13$     | de   | $0.84\pm0.06$ | de     | 4.01±0.09        | ab                   | 4.28±0.24     | а      | 23.30±0.12       | cd             | 24.85±0.58       | bc                    | $2.16\pm0.07$    | а     | $2.09\pm0.04$   | ab  |
| Water (Control)                 | $0.74\pm0.19$     | de   | $0.59\pm0.01$ | ef     | 3.22±0.44        | c-e                  | $2.84\pm0.08$ | de     | $25.04 \pm 0.61$ | bc             | 30.06±0.13       | а                     | $1.89\pm0.06$    | cd    | $1.88\pm0.02$   | d   |
| Mean                            | 0.74              | b    | 1.38          | а      | 3.16             | а                    | 3.22          | а      | 21.42            | b              | 23.84            | а                     | 2.05             | а     | 1.94            | b   |
| <sup>z</sup> Mean values follow | ed by a letter    | in c | ommon were    | not    | significantly o  | liffer               | ent accordin  | g to 1 | Duncan's mu      | ltipl          | e range test (p  | <0.(                  | 05). Mean val    | lue ± | standard er     | ror |

Previous works, in agreement with our results, found that foliar spray with calcium and potassium silicate enhanced average fruit weight, fruit firmness, marketability, and total yield in strawberry (EL-Sayed et al., 2023). Nada (2020) reported a significant increment in fruit weight, fruit firmness, and early and total yield in strawberry due to foliar spraying with potassium silicate. Rozbiany and Taha (2020) found that fruit fresh weight and yield/plant were significantly improved due to foliar Spraying with 500 ppm of calcium in strawberry cv. Festival. Also, there was a positive impact on early and total yield in tomatoes after foliar treatment with salicylic acid and potassium silicate (EL-Sayed et al., 2023). Youssef et al., (2017) tested the effect of eight concentrations of salicylic acid and found that salicylic acid at 4.0 mM produced the biggest average fruit weight and the highest early and total yield.

## Effect of bio-stimulants, cultivars, and their interaction on fruit quality traits

Data in Figure 6 exhibited that significant differences were found between bio-stimulants on strawberry plants for TSS, titratable acidity, ascorbic acid and anthocyanin

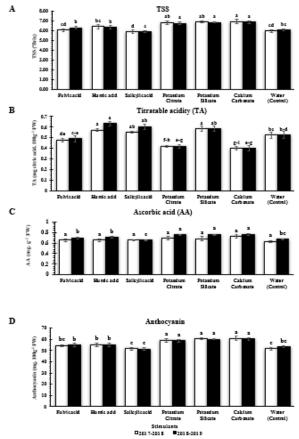
concentration. Concerning TSS, potassium citrate, potassium silicate, and calcium carbonate demonstrated the highest content of TSS in both seasons. Concerning titratable acidity, humic acid, salicylic acid, and potassium silicate showed the highest value of titratable acidity in fruits in both seasons, while potassium citrate and calcium carbonate decreased the titratable acidity value. Concerning ascorbic acid, no significant differences were found among bio-stimulants in the first season. Meanwhile, potassium citrate, potassium silicate, and calcium carbonate displayed the highest ascorbic acid in fruits in the second season. Regarding anthocyanin concentration, calcium carbonate, potassium citrate and potassium silicate in both seasons exhibited the highest significant value.

Concerning the interaction between cultivars and biostimulants, Table 6 displayed that significant differences were found among the effects of the bio-stimulants in the two cultivars for titratable acidity, TSS, anthocyanin in both seasons and ascorbic acid in second season only.

Potassium citrate, potassium silicate in both cultivars and calcium carbonate in Fortuna cultivar showed the highest TSS content in first season, while all bio-stimulants enhanced the TSS content in Fortuna cultivar, except salicylic acid, while potassium silicate and calcium carbonate in both seasons showed the highest TSS in Festival cultivar, and potassium citrate ranked second in TSS in Festival cultivar in the second season. Concerning titratable acidity, humic acid in both cultivars in addition to salicylic acid and potassium silicate in Festival cultivar during both seasons showed the highest value of titratable acidity in fruits. Concerning ascorbic acid in second season, potassium silicate and calcium carbonate in both cultivars, and humic acid and potassium citrate in Fortuna cultivar exhibited the highest ascorbic acid concentration in fruits during the second season. Regarding anthocyanin concentration in fruits, potassium Silicate in the two cultivars, in addition to potassium citrate and calcium carbonate in Fortuna cultivar exhibited the highest anthocyanin concentration in the first season, while all bio-stimulants increased the content of anthocyanin in Fortuna, except salicylic acid in the second season, but in festival cultivar, potassium silicate, and calcium carbonate displayed the highest concentration of anthocyanin.

Cultivars displayed a significant effect on titratable acidity and anthocyanin concentration in the both seasons and TSS and ascorbic acid in the second season.

Similar findings by Al-Qadi and Alimam (2023) evaluated three concentrations of humic acid (0, 3, and 6 ml  $L^{-1}$ ) and found that 3 ml  $L^{-1}$  increased total acidity content in strawberry fruits. Kazemi et al. (2014) assessed the effect of two concentrations of humic acid (15 and 25 mL  $L^{-1}$ ) on fruit quality and found that both levels of humic significantly increased TSS, vitamin C, and acidity %. Rozbiany and Taha (2020) reported that foliar spraying with calcium significantly increased TSS, vitamin C, and acidity %. EL-Sayed et al. (2023) tested the effect of foliar spray with potassium silicate and calcium on the chemical fruit quality traits of strawberry fruits. Calcium significantly improved anthocyanin concentration, while potassium silicate had no significant effect on this trait.



Columns with the same letter represent values that are not significantly different according to Duncan's multiple range test (p < 0.05). Vertical bars represent ± standard error of the mean.

Fig. 6. Fruit chemical composition (A: TSS; B: titratable acidity; C: ascorbic acid; and D: anthocyanin) of two strawberry cultivars treated with some bio-stimulants in greenhouse conditions during the 2017-2018 and 2018-2019 winter seasons.

| Table 6. Fruit chemical composition of strawberry cultivars Fortuna and Festival treated with some bio-stimulants in |
|--|
| greenhouse conditions during the 2017-2018 and 2018-2019 winter seasons.   |

| Cultivars (C)      | Т             | SS        |     | Titra            | table | e acidity               |     |            |                           | ic acid          |    | Anthocyanin                 |                  |     |  |  |
|--------------------|---------------|-----------|-----|------------------|-------|-------------------------|-----|------------|---------------------------|------------------|----|-----------------------------|------------------|-----|--|--|
| Cuuvars (C)        | (°E           | Brix)     |     | (mg citric       | acid  | . 100g <sup>-1</sup> FW | 0   | (m         | <b>g. g</b> <sup>-1</sup> | <sup>I</sup> FW) |    | (mg. 100g <sup>-1</sup> FW) |                  |     |  |  |
| Stimulants(S)      | Fortuna       | Festiva   | l   | Fortuna Festival |       |                         |     | Fortuna    | L                         | Festival         |    | Fortuna                     | Festival         | I   |  |  |
|                    |               |           |     |                  |       |                         | 201 | 7-2018     |                           |                  |    |                             |                  |     |  |  |
| Fulvic acid        | 624±021 be    | 5.89±0.23 | e   | 0.45±0.027       | ef    | 0.48±0.022              | de  | 0.65±0.047 | ab                        | 0.66±0.011       | ab | 56.77±0.37                  | c-e 5221±0.83    | ef  |  |  |
| Humic acid         | 6.70±0.17 ab  | 6.11±036  | be  | 0.56±0.012       | ab    | 0.57±0.024              | а   | 0.68±0.045 | ab                        | 0.63±0.024       | ab | 57.19±0.46                  | be 53.49±3.17    | d-f |  |  |
| Salicylic acid     | 5.78±0.31 e   | 595±024   | de  | 0.50±0.005       | cd    | 0.55±0.007              | ab  | 0.67±0.020 | ab                        | 0.64±0.017       | ab | 52.79±1.55                  | ef 50.46±1.31    | f   |  |  |
| Potassium Citrate  | 6.98±0.16 a   | 6.65±031  | ас  | 0.39±0.014       | h-j   | 0.42±0.010              | fh  | 0.71±0.051 | ab                        | 0.67±0.048       | ab | 60.08±0.95                  | a-c 58.19±2.72 1 | bd  |  |  |
| Potassium Silicate | 6.74±0.08 ab  | 7.01±0.18 | а   | 0.45±0.005       | e-g   | 0.59±0.013              | а   | 0.65±0.056 | ab                        | 0.72±0.061       | ab | 58.99±0.70                  | a-c 61.98±1.49   | ab  |  |  |
| Calcium Carbonate  | 723±035 a     | 6.63±0.17 | a-d | 0.35±0.016       | j     | 0.40±0.016              | g-i | 0.71±0.072 | ab                        | 0.74±0.019       | а  | 63.88±2.98                  | a 57.97±1.47 1   | bd  |  |  |
| Water (Control)    | 597±023 се    | 594±0.16  | de  | 0.36±0.007       | ij    | 0.53±0.005              | bc  | 0.62±0.033 | b                         | 0.65±0.014       | ab | 52.21±2.04                  | ef 51.04±1.07    | f   |  |  |
| Mean               | 652 a         | 631       | а   | 0.43             | b     | 0.50                    | а   | 0.66       | а                         | 0.67             | а  | 57.41                       | a 55.04          | b   |  |  |
|                    |               |           |     |                  |       |                         | 201 | 8-2019     |                           |                  |    |                             |                  |     |  |  |
| Fulvic acid        | 6.67±0.07 ab  | 595±0.10  | с   | 0.45±0.043       | d-f   | 0.49±0.049              | c-e | 0.74±0.008 | bc                        | 0.66±0.011       | e  | 58.14±0.62                  | ab 52.06±0.89    | d   |  |  |
| Humic acid         | 6.73±0.03 ab  | 592±0.17  | с   | 0.55±0.018       | ас    | 0.63±0.018              | а   | 0.75±0.003 | а-с                       | 0.66±0.019       | e  | 58.92±0.25                  | ab 51.32±1.56    | d   |  |  |
| Salicylic acid     | 5.93±0.13 c   | 5.86±0.19 | с   | 0.53±0.035       | bd    | 0.61±0.031              | ab  | 0.66±0.015 | e                         | 0.65±0.021       | e  | 51.49±1.21                  | d 51.26±1.64     | d   |  |  |
| Potassium Citrate  | 7.05±0.03 a   | 6.47±0.19 | b   | 0.39±0.019       | fg    | 0.42±0.033              | e-g | 0.79±0.04  | а                         | 0.72±0.021       | cd | 61.69±0.30                  | a 56.07±1.74     | bc  |  |  |
| Potassium Silicate | 6.78±0.03 ab  | 691±0.10  | а   | 0.44±0.009       | d-f   | 0.59±0.018              | ab  | 0.76±0.004 | а-с                       | 0.77±0.011       | ab | 59.44±0.31                  | ab 60.45±0.86    | а   |  |  |
| Calcium Carbonate  | : 7.02±0.22 a | 6.72±0.14 | ab  | 0.35±0.043       | g     | 0.40±0.023              | e-g | 0.78±0.025 | ab                        | 0.75±0.016       | ас | 61.40±1.96                  | a 59.15±1.31     | ab  |  |  |
| Water (Control)    | 6.07±0.19 c   | 6.02±0.05 | с   | 0.36±0.019       | fg    | 0.53±0.013              | bd  | 0.68±0.021 | de                        | 0.67±0.005       | e  | 53.60±1.74                  | cd 52.78±0.44    | cd  |  |  |
| Mean               | 6.60 a        | 626       | b   | 0.43             | b     | 0.52                    | a   | 0.73       | Α                         | 0.69             | b  | 57.81                       | a 54.72          | b   |  |  |

<sup>2</sup>Mean values followed by a letter in common were not significantly different according to Duncan's multiple range test (p <0.05). Mean value ± standard error:

The highest concentration of vitamin C was achieved using 0.6% potassium silicate, also 1% and 2% of calcium treatment increased this content. Calcium treatment and 0.6 % potassium silicate application increased acidity %, but TSS was not influenced in both seasons. Nada (2020) tested four concentrations of potassium silicate  $(0, 0.2, 0.4, and 0.6 \text{ g L}^{-1})$ on strawberry plants, and found that all concentrations caused significant increments in TSS, acidity%, vitamin C, and anthocyanin concentration in fruits. In contrast, the interaction effects of foliar application of humic acid and two cultivars (Camarosa and Nabila) on quality parameters. TSS, titratable acidity, and vitamin C were found non-significant (Chakraborty et al., 2023), also Ullah et al. (2017) tested four levels of humic acid (0, 1.5, 3, and 4.5 ml L<sup>-1</sup>) on strawberry plants and found that humic acid showed a non-significant effect on the titratable acidity of fruit (Ullah et al., 2017).

#### CONCLUSION

The impact of different bio-stimulants i.e., humic acid, fulvic acid, salicylic acid, potassium citrate, potassium silicate, and calcium carbonate on plant vigor, yield, and quality attributes in two strawberry cultivars (Fortuna and Festival) in a plastic greenhouse were studied. Calcium carbonate and potassium silicate enhanced all most studied traits except dry matter, alkaloids in both stimulants, antioxidant activity, and titratable acidity in calcium carbonate, and their effects were different among the cultivars.

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## زيادة النمو والمحصول والمركبات النشطة بيولوجيا فى الفراولة بواسطة المنشطات الحيوية

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#### الملخص

حظيت المنشطات الحبوية باهتمام عالمي نظرًا لتأثير اتها المغيدة على نمو وتطور العديد من المحاصيل. أجريت التجربة خلال موسمي الشتاء لتقييم ومقارنة تأثير منشطات جيوية مختلفة على قوة النمو والمحصول وصفات الجودة في صنفين من الفراولة (فررتونا وفيستيفل). وكانت المعاملات: 1) حامض الهيوميك 15% (10 مللي/لتر)، و2) حامض الفولفيك 15 % (10 مللي/لتر)، و3) سترات بوتلسيوم (2 جرام/لتر)، و4) سيليكات البوتلسيوم (2 جرام/لتر)، و5) حامض السلسيليك (1 جرام/لتر)، و6) كربونات الكلسيوم (2 جرام/لتر)، و7) كنتر ول (مام). كل المعاملات كانت رش على الاوراق ماعدا الهيوميك كان اضافة ارضية. وتمت معاملة نباتات الفراولة اسبو عيا حيث تمت اول معاملة بعد 15 يوم من الشتل وتم تكرار ها كاتر ول (مام). كل المعاملات كانت رش على الاوراق ماعدا الهيوميك كان اضافة ارضية. وتمت معاملة نباتات الفراولة اسبو عيا حيث تمت اول معاملة بعد 15 يوم من الشتل وتم تكرار ها 10 مرات. أفضل أداء كان لسيليكات البوتاسيوم وكربونات الكالسيوم حيث أن كربونات الكالسيوم حيث أن كريونات الكالسيوم في الأوراق بينما حسنت سيليكات البوتاسيوم كل الصفات ماعدا المادة الجافة والقريدات الكلية في الأوراق. أظهرت سترات البوتاسيوم اعلى قيمة للفلافونيدات الكلية ونشاط مضادات الأكسدة في الأوراق بينما حسنت سيليكات البوتاسيوم كل الصفات ماعدا المادة الجافة والقريدات الكلية في الأوراق. أظهرت سترات البوتاسيوم اعلى قيمة للفلافونويدات في الأوراق وصلابة الأمار والمادة الصلبة الذائبة الكلية. الهيوميك أظهر أعلى نشاط لمضادات الأكسدة في الأوراق والحموضة المعايرة. الفولي أظهر أعلى معار لموراق وصلابة الثائير والمادة الصلبة الذائبة الكلية. الهيرميك أظهر أعلى نشاط لمضادات الأكسدة في الأوراق والحوضة المعايرة العراقيري مي والمادة الصلبة الذائبة الكلية. الهيرمية أطهر أصلور أعلى نشاط لمضادات الأكسدة في الأوراق والحموض المعاني في تلفي الغربي التعربي في المعاني في علق بالتأثير والمادة الصلبة الذائبة الكلية. المعرر أعلى نشاط لمضادات الأكسدة في الأوراق والحموضة المعاري قلي أعلى مناور الق والماد مصدات الأكسدة في الأوراق والمعال على والمعنات في تلفير الأصداف كان هذاك أظهر أعلى محتوى من الكرو وماعدا حد الأوراق النابق والماط مصندات الأكسدة في الأوراق والمعصول الكلي ومتوسط والمائية الكلية وفي لمين ج في الموسة المراور ال الموراق النوراي

الكلمات المفتاحية: الفراولة، المنشطات الحيوية، الإنتاجية، الجودة