The Biological Response of the Stevia Rebaudiana Bertoni to Bio-Enriched and Nano Amino Acids
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Abstract. A field experiment carried out in the wooden canopy - Department of Horticulture and Gardening Engineering - College of Agriculture - University of Anbar to testing the response of Stevia rebaudiana bertoni to spraying with - Bio-Enriched and nano Proline, Where three levels of Nano- Bio-Enriched (0mg, 50mg and 100mg) were used, three levels of nano Proline were used (0mg, 100mg and 200mg). The results were recorded showed that the treatments were sprayed with the Nano- Bio-Enriched (100mg) were significantly superior with the highest ratios of the stem’s diameter (ml), Number of branches per plant and Chlorophyll content in the leaves.

1. Introduction
Stevia (Scientific name Stevia rebaudiana bertoni). A sweet, fragrant herb with amazing nutritional properties. Its sweetness returns to its nature, which contains sugars molecules and compounds, and its taste is distinctive with a little bitterness and resembles the taste of licorice, and the more good it is, the less bitterness in it [1]. Its sweetness is not similar to that of regular sugar or artificial sugar, but it is acceptable and a person may need to get used to its taste [2]. The plant is a frost-sensitive perennial herb. Its leaves are high content in sugar (twenty to thirty times sweeter than sugar cane) [3]. It is a subtropical plant type and that stevia is originally grown in the mountain regions of Paraguay and Brazil, and has been used in its habitat for centuries as a native green tea herb and a herb that helps treat the digestive system and heal topical wounds [4]. Her first harvest in her native South America dates back to 1908, and farms were established in North America in 1921 [5].

Stevia contains many nutritional and health benefits. Stevia is a very sweet herb, making it calorie-free, so doctors advise to eat it instead of chemical sweeteners. to avoid the damage caused by chemical sugars [4]. Stevia is rich in nutrients such as protein, calcium, phosphorous, sodium, magnesium, zinc, vitamins A and C. Stevia grows successfully from seed in most soils, but prefers sandy soils rich in organic matter as well as clay soil, and this plant needs an exceptional system of drainage, and it prefers slightly acidic soil [6]. Long spring and summer days lead to leaf growth, while short days encourage flower growth. Stevia plants can be grown indoors in the winter with the use of fluorescent light for 14-16 hours per day. Small pots 8-10 cm in diameter can also be used. Cuttings can be taken from these plants during the winter to give new plants for the spring [7].

Numerous studies have indicated the positive role of proline acid in improving the growth and yield characteristics of the plant. Study showed that foliar spraying with proline acid depends on the type and variety of the plant, the time of addition, and the appropriate concentration, which has a role in improving growth characteristics and yield. [8] also showed in his study that proline acid had a significant effect on the characteristics of vegetative...
growth, as it led to an increase in plant height, leaf area and dry weight of the vegetative group when it was used as a spray on plants at different concentrations, especially at a concentration of 20 mg per liter under different levels stress [9].

2. Materials and Methods
A field experiment was carried out at the College of Agriculture, University of Anbar, in order to test the effectiveness of nano-biological fertilizer and nano proline in the biological activities of stevia plants. Stevia was grown in plastic pots of 40 x 50 cm dimensions. Organic compost, sand and peat moss were mixed in equal proportions in pots. A solution stock was prepared from nano-biological fertilizer and Proline acid with a weight of 1 g and dissolved in 1000 ml of distilled water to obtain a base solution at a concentration of 1000 parts per million mg. Liters, then the required concentrations of Nano- Bio-Enriched (0mg, 50mg and 100mg) and nano Proline (0mg, 100mg and 200mg) were prepared. Liter. According to the law of dilution, the plants were sprayed in the early morning until complete wetness, after the 30 days the plants were sprayed with nan organic fertilizer and nano proline [10]. The control treatments were sprayed with distilled water [11].

The experiment was carried out, according to the design of Randomized Complete Block Design (RCBD) within three replicates for vegetative and chemical properties.

3. Results and Discussion
3.1 Stem’s diameter for the plant
Data in table 1 expose the stem’s diameter for the plant that were sprayed with the nano fertilizer compared with the treatments that weren’t sprayed with the nano fertilizer, where the highest rate of the stem’s diameter was recorded when plants sprayed with the highest percentage of fertilizer (6.31) with a significant difference from the other treatments reached to (5.01), while the lowest rate of the stem’s diameter (3.75) was recorded at the plants that weren’t sprayed with the biological nano fertilizer along the growing season, which recorded a significant decrease from the other study treatments. As for the nano Proline, a clear response to the stem’s diameter was recorded, where the highest rate of the stem’s diameter was recorded in the plants that were sprayed with the highest rate of nano Proline (9.01) where reached (6.92) with a significant difference from the other study treatments. The lowest the stem’s diameter was recorded at the comparison treatments reached (3.75). The interaction between the two factors of the experiment led to a significant impact on the stem’s diameter, where the highest rate of the stem’s diameter was recorded at the combination (nano fertilizer and nano Proline) with the stem’s diameter was (12.45), while the lowest rate of the stem’s diameter was recorded in plants that weren’t sprayed with nano fertilizer and the nano Proline, where the stem’s diameter reached (3.75) with a significantly reduced compared with the other treatments.

Table 1) effect of nano organic fertilizer and the nano proline on stem’s diameter for the plant.

| Nano-fertilizer concentrations (ppm) | Nano proline concentrations (ppm) | Mean |
|-------------------------------------|-----------------------------------|------|
| 0                                   | 0                                 | 3.75 | 6.92 | ED.101 | 6.56 |
| 50                                  | 50                                | 5.01 | 7.75 | 10.22  | 7.66 |
| 100                                 | 100                               | 6.31 | 8.70 | 12.45  | 9.15 |
| LSD (0.05)                          | Nano-fertilizers = 0.533          |      |      |        |
|                                     | Nano proline = 0.533              |      |      |        |
|                                     | Nano-fertilizers × Nano proline = 0.922 |      |      |        |

3.2 Number of branches per plant
Results of statistical analysis in table 2 clarify the number of branches per plant that were sprayed with the nano fertilizer compared with the treatments that weren’t sprayed with the
nano fertilizer, where the highest rate of the branches’ number was recorded when plants sprayed with the highest percentage of fertilizer (4.33) with a significant difference from the other treatments reached to (3.99), while the lowest rate of the branches’ number (2.86) was recorded at the plants that weren’t sprayed with the biological nano fertilizer along the growing season, where recorded a significant decrease from the other study treatments. As for the nano Proline, a clear response to the number of branches was recorded, where the highest rate of the branches’ number was recorded in the plants that were sprayed with the highest rate of nano Proline (7.22) where reached (5.88) with a significant difference from the other study treatments. The lowest rate of the branches’ number was recorded at the comparison treatments reached (2.86). The interaction between the two factors of the experiment led to a significant impact on the number of branches, where the highest rate of the branches’ number was recorded at the combination (nano fertilizer and nano Proline) was (8.34), while the lowest rate of the branches’ number was recorded in plants that weren’t sprayed with nano fertilizer and the nano Proline, where the number of branches reached (2.86) with a significantly reduced compared with the other treatments. These results are in line with [10].

Table (2) effect of nano organic fertilizer and the nano proline on number of branches per plant.

| Nano-fertilizer concentrations (ppm) | Nano proline concentrations (ppm) | Mean  |
|-------------------------------------|----------------------------------|-------|
| 0                                   | 0                                | 2.86  |
| 50                                  | 100                              | 3.99  |
| 100                                 | 200                              | 4.33  |
| LSD                                 | Nano-fertilizers = 0.3652        |       |
| (0.05)                              | Nano proline = 0.3652            |       |
|                                     | Nano-fertilizers × Nano proline = 0.6326 |       |

3.3 Chlorophyll content in the leaves
The information in table 3 clarify the measurement of chlorophyll content in the leaves that were sprayed with the nano fertilizer compared with the treatments that weren’t sprayed with the nano fertilizer, where the highest rate of chlorophyll was recorded when plants sprayed with the highest percentage of fertilizer (6.05) with a significant difference from the other treatments reached to (4.04), while the lowest rate of chlorophyll (3.89) was recorded at the plants that weren’t sprayed with the biological nano fertilizer along the growing season, which recorded a significant decrease from the other study treatments. As for the nano proline, a clear response to percentage of chlorophyll content in the leaves recorded, where the highest rate of percentage of chlorophyll was recorded in the plants that were sprayed with the highest rate of nano proline (5.86) where reached (4.77) with a significant difference from the other study treatments. The lowest percentage of chlorophyll was recorded at the comparison treatments reached (3.89). The interaction between the two factors of the experiment led to a significant impact on the chlorophyll, where the highest rate of percentage of chlorophyll content was recorded at the combination (nano fertilizer and nano Proline) was (8.75), while the lowest rate of chlorophyll was recorded in plants that weren’t sprayed with nano fertilizer and the nano proline, where the chlorophyll reached (3.89) with a significantly reduced compared with the other treatments. These results are in agreement with [12].

Table (3) effect of nano organic fertilizer and the nano proline on the chlorophyll content in the leaves.

| Nano-fertilizer | Nano proline | Mean  |
|-----------------|--------------|-------|
|                 |              |       |

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4. Conclusion
It was concluded from the above study that the nan organic fertilizer and nano proline have clear significant effects on all biological and physiological plant activities, as the highest increase in the studied traits appeared at the high level of nan organic fertilizer and nano proline, while the lowest was recorded at the comparison treatment.

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