Effect of Phenylalanine, Jasmonic Acid and Biofertilizer on Growth, Yield and Anthocyanin Pigments of Roselle Calyces

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Abstract

Experiment was conducted during summer season of 2021 to study the effect of foliar applications of Phenylalanine (Phe), Jasmonic acid (JA), Biofertilizer (Bio) and their combinations (Phe+JA, Phe+Bio, JA+Bio, Phe+JA+Bio and control) on some growth, yield parameters and anthocyanin compounds on Roselle calyces \textit{Hibiscus sabdariffa} L. The treatments were distributed on Randomized Completely Block Design in three replicates, means were compared according to the Least Significant Difference test (LSD) at probability level of 0.05. The results show that growth parameters (plant height, branches number, leaf area and chlorophyll) and yield parameters (fruits number, fresh weight calyces, fresh yield calyces and dry yield calyces) were increased due to foliar application Phe+JA+Bio, while the combination treatment Phe+Bio gave significant effects on vitamin C, total anthocyanin, Gossypetin, Sabdarta, Hibescitine, Delphidine and Cyanidin of calyces reached at 201.5, 184.5, 29.3, 31.2, 57.4, 35.4, 27.8 (mg.100g) respectively.

Keyword: Roselle, Biofertilizer, Phenylalanine, Jasmonic acid, Anthocyanins.

1.\textbf{Introduction}

Roselle (\textit{Hibiscus sabdariffa} L.) is an annual plant belongs to family Malvaceae, It is mainly cultivated for purpose of obtaining the red calyces (fleshy sepals), which are rich on anthocyanin pigments and vitamin c [1,2]. This plant uses in many treated in herbal medicine, some of them employing as antiseptic, digestive, diuretic, cough, debility, dyspepsia, dysuria, fever, hangover, emollient and purgative [3]. Many researchers refer to the benefit of roselle for prevented disease or complementary treatments for cancer, heart ailments, hypertension, neurosis, scurvy, anti-inflammatory, antihypertensive and antimutagenic [4,5]. The active parts of Roselle (calyces) uses in many forms such as tea pies, desserts, sauces, natural coloring agents in food industries, emulsifier for carbonated drinks and cosmetics [6]. The use of biostimulants is one of the most important trends in the recent study, as vitamins, hormones and microorganisms are used to encourage plant growth and improve cellular metabolism [7]. The organic stimulants reduce the need for fertilizers, improve plant growth and increase productivity [8].

A few investigations have suggested that biostimulants had positive effects on growth, yield and quality calyces of roselle, one of these methods was add biofertilizer, which increased the growth, yield and concentrations of vitamin c and anthocyanins pigments of roselle, by provided or improving readiness of nutrients in soil [9,10]. Biofertilizer consist of Azotobacter mainly, this microorganisms are non-symbiotic, operated nitrogen fixation in soil, which increases nitrogen abundance and improved the level of phytohormones which promotion plant growth [11,12].

Phenylalanine is a vital stimulant, it is basis for the metabolism of many active compounds such as phenols, flavonoids and terpenes, which makes it an important role in promoting auxins and gibberellins, as well as its role as a source of nitrogen necessary for building proteins and enzymes that promote plant growth and yield [13]. The researchers [14] found that treating roselle plants with phenylalanine led to an increase in growth indicators (plant height, branch number, total chlorophyll and total dry weight), yield components (fruit number and fresh and dry weight sepals yield per plant) and anthocyanin pigment content. The [15] reported that growth and yield of \textit{Trigonella foenum-graecum} L. plants parameters increased significantly with increasing spraying phenylalanine concentrations.

Plant growth regulators controlled of plant’s physiological and biochemical processes, mainly crop growth, development, flowering, fruit set and mineral uptake [16,17], addition antioxidant activity, ingredient compounds and anthocyanin pigments [18]. Jasmonic acid (JA) stimulate growth, nutrient transfer, photosynthesis rat and metabolic anthocyanin pigments [7]. Some researchers have noticed catalyst relationship between concentration of jasmonic acid and synthesis of anthocyanin.
pigments in several plants, including [19] in *Glycine max* Merr., [20] in *Kalanchoe blossfeldiana* L. [21] in *Raphanus sativus* L., these researchers found an increase in concentrations of anthocyanin pigments when treated plants with jasmonic acid. This study was done to evaluate the growth, yield and anthocyanin pigments responses of Roselle cultivar “Local Egyptian” to some biostimulants factors and their combinations to determine the optimum treat that can be recommended to improve quality, quantity production of calyces and its contain of anthocyanin pigments.

2. Materials and Methods

This experiment was conducted in Private farm during the summer growing season at 2021 in Babylon governorate, seeds of Roselle cultivar “Locale”, it was planted at 20 March 2020 after testing the germination percentage in seed technology laboratory at the Agriculture college/Al-Qasim Green University, total experimental units were 30, every unit 6.75 m² (3 × 0.75 m) consist of 4 lines, distance between plants 0.5 m, total plants in unit are 24, the soil texture was loamy with properties observed table (1). The treatments were distributed at Randomized Completely Block Design in three replicates, means were compared according to the Least Significant Difference test (LSD) at probability level of 0.05.

| Sample depth (cm) | pH | Organic Matter (%) | Ec (dsm⁻¹) | Total Nitrogen (%) | Available Phosphorus (mgkg⁻¹) | Available Potassium (mgkg⁻¹) | Silt (%) | Clay (%) | Sand (%) | Soil Texture |
|-------------------|----|--------------------|------------|-------------------|-------------------------------|-------------------------------|----------|----------|----------|--------------|
| 0-30              | 7.8| 1.93               | 1.2        | 2.1               | 8.2                           | 220                           | 44.5     | 29.4     | 26.1     | Silt-Clay    |

The experiment consisted of spraying stimulants factors were: phenylalanine (Phe) at concentration 50 ppm, Jasmonic acid (JA) at concentration 400 ppm, biofertilizer (Bio) contain (*Mycorrhiza horizontal*, *Bacillus subtilis* 10%, Humin acid 70%, seaweed extract 5% and organic mater 65%) at level 5 Kg.h⁻¹ (mixing with soil at cultivation) and their combinations (Phe+JA, Phe+Bio, JA+Bio, Phe+JA+Bio and control), all treatments were applied by foliar spraying at four doses at two week-intervals started after 14 days of planting, also the control treat was sprayed with distilled water only. The experiment done to study the impact of spraying stimulants factors and their combinations on some growth parameters (plant height, branch number, leave area and Chlorophyll), which measured at the end stage of vegetative growth, yield components (fruits number and Yield Parameters: fruit number, fresh weight calyx, yield fresh weight calyces, yield dry weight calyces) which determined at the end of flowering stage in 30 October 2020 and chemical constituents of calyces of Roselle: Vitamin C, Total Anthocyanin, Gossypetin, Sabdarite, Hibescitine, Delphidine and Cyanidin, the chemical constituents were measured after the final harvest, collected the fruits and sampling was taken randomly in average 20 fruits for each experimental unit, it was air-dried for 14 days and crushed into powder with electrical grinder, the chemical constituents were separated by using high performance liquid chromatography (HPLC) device, this analysis was conducted by using the method which described by [22-24]. The basic compounds were separated by flow liquid chromatography (FLC), where the column under ideal conditions with specifications of C18 (250 mm × 4.6 mm I.D., 3 μm), length 50 mm and a diameter of 2 mm with a flow rate of 1 mL/min at room temperature. The mobile phase consists of solvent A containing 0.1% of trifluoroacetic acid (TFA) in anion water. The solvent B consists of 0.1% of formic acid, The elution quickly flow 1.2 ml.min were seized detector at the wavelength of 280 nm [23].

3. Results and Discussion

3.1 Growth Parameters

Results in table (2) indicate that treat Phe+JA+Bio archived significant effects (P<0.05) of Roselle plants during vegetative growth on parameters plant height, branches number, leaves area and total chlorophyll which resulted to be 177.3 cm, 15.7 branches, 1.396 cm² and 39.8 SPAD respectively, compared with control which recorded 151.3 cm, 8.1 branches, 0.983 cm² and 25.6 SPAD respectively. This achievement on growth parameters may be due to the role of biofertilizer in providing the necessary nutrients for growth and development of plants [25,26], along with effect of amino acid phenylalanine, which is a suitable substitute for necessary nitrogen for construction of amino acids and various proteins, as well as the role of jasmonic acid, which is a growth hormone and a vital carrier of chemical signaling system, this formed a good compatibility and performance to increase growth parameters of plant.
osure area to light with oligo compounds as intermediate compounds or westive compounds, especially flavonoids, including e significantly on treat Phe + JA , or perhaps the increase is due to the role of phenylalanine. Bio resulted in highest values of active constitutes: vitamin C, Total Anthocyanins and their compounds in increasing products of Shikimic acid pathway, that result in most of the act product thereof, thus the increase is conjugated between them are secondary metabolites, that are directly related to primary metab that have

201.5, 184.5, 29.3, 31.2, 57.4, 35.4, 27.8 (mg.100g) respectively. Anthocyanin, Gossypetin, Sabdartine, Hibiscetin, Delphidine and Cyanidin content in dry calyces, which evaluated to be 124.3, 142.9, 21.5, 23.6, 46.1, 27.8, 20.2 (mg.100g)

3.2 Yield Parameters

Also table (1) explain done superiority significant of treat Phe+JA+Bio in fruits number, fresh weight calyx, yield fresh weight calyces and yield dry weight calyces reached 45.8 frute.plant \(^{-1}\), 1.398 g.plant \(^{-1}\), 64.03 g.plant \(^{-1}\) and 4.873 g.plant \(^{-1}\) respectively, compared with control which gave 24.1 frute.plant \(^{-1}\), 1.124 g.plant \(^{-1}\), 27.09 g.plant \(^{-1}\) and 2.463 g.plant \(^{-1}\) respectively. The increase in yield parameters may be due to increase in rate of photosynthesis and accumulation of dry matter as a result of the increase in vegetative growth indicators, which provided an effective exposure area to light with high chlorophyll concentrations, as well as availability of requirements for increasing the building of hormones and enzymes by improving the nutritional system of plant mediated by biofertilizer and phenylalanine.

3.3 Anthocyanin Pigments

Data presented in table (3) show that biostimulants effect in concentrations of anthocyanin pigments in dry calyces of Roselle plant, these effects were significantly on treat Phe+Bio resulted in highest values of active constitutes: vitamin C, Total Anthocyanin, Gossypetin, Sabdartine, Hibiscetin, Delphidine and Cyanidin content in dry calyces, which evaluated to be 201.5, 184.5, 29.3, 31.2, 57.4, 35.4, 27.8 (mg.100g) respectively, compared with control treat (spray with distilled water only) which have 124.3, 142.9, 21.5, 23.6, 46.1, 27.8, 20.2 (mg.100g) respectively. The increase in anthocyanins pigments, which are secondary metabolites, that are directly related to primary metabolic compounds as intermediate compounds or west product thereof, thus the increase is conjugated between them [15], or perhaps the increase is due to the role of phenylalanine in increasing products of Shikimic acid pathway, that result in most of the active compounds, especially flavonoids, including anthocyanins and their compounds [13].

| Treatments | PH | BN | LA | Ch | FN | FWC | YFWC | YDWC |
|------------|----|----|----|----|----|-----|-------|-------|
| Control    | 151.3 | 8.1 | 0.983 | 25.6 | 24.1 | 1.124 | 27.09 | 2.463 |
| Phe        | 156.4 | 8.7 | 1.056 | 26.3 | 28.3 | 1.141 | 32.29 | 2.933 |
| JA         | 154.6 | 8.3 | 0.998 | 25.8 | 26.7 | 1.138 | 30.38 | 2.755 |
| Bio        | 158.4 | 10.3 | 1.103 | 30.1 | 32.5 | 1.189 | 38.64 | 3.512 |
| Phe + JA   | 162.9 | 11.2 | 1.123 | 29.5 | 33.8 | 1.209 | 40.86 | 3.713 |
| Phe + Bio  | 167.5 | 12.8 | 1.292 | 31.1 | 35.4 | 1.336 | 47.29 | 4.297 |
| JA + Bio   | 173.6 | 12.3 | 1.276 | 30.6 | 34.1 | 1.289 | 43.95 | 3.994 |
| Phe + JA + Bio | 177.3 | 13.7 | 1.326 | 31.8 | 36.8 | 1.398 | 51.45 | 4.673 |

Growth Parameters: plant height cm (PH), Branch No. (BN), Leave area cm² (LA), Chlorophyll mg g⁻¹ FW (Ch) and Yield Parameters: fruit No. (FN), fresh weight calyx g (FWC), yield fresh weight calyces g.plant (YFWC), yield dry weight calyces g.plant (YDWC).

Table 2. Effect of some Biostimulants treats on growth and yield parameters on Roselle calyces.

3.2 Yield Parameters

| Treatments | Vitamin C | Total Anthocyanin | Gossypetin | Sabdartine | Hibiscetin | Delphidine | Cyanidin |
|------------|-----------|-------------------|-----------|------------|------------|------------|----------|
| Control    | 124.3     | 142.9             | 21.5      | 23.6       | 46.1       | 27.8       | 20.2     |
| Phe        | 163.2     | 154.3             | 23.7      | 26.4       | 49.7       | 29.3       | 21.8     |
| JA         | 155.8     | 151.3             | 23.4      | 28.1       | 48.6       | 27.4       | 20.5     |
| Bio        | 139.2     | 145.9             | 22.8      | 25.7       | 47.3       | 27.1       | 20.8     |
| Phe + JA   | 175.8     | 162.6             | 24.5      | 28.5       | 52.4       | 31.4       | 22.4     |
| Phe + Bio  | 201.5     | 184.5             | 29.3      | 31.2       | 57.4       | 35.4       | 27.8     |
| JA + Bio   | 184.6     | 169.1             | 25.3      | 29.4       | 53.9       | 33.6       | 24.1     |
| Phe + JA + Bio | 198.4 | 179.8             | 27.5      | 30.8       | 56.8       | 34.7       | 26.5     |

Table 3. Effect of some Biostimulants treats on anthocyanin compounds (mg.100g) of Roselle calyces.
Conclusion

The foliar application of phenylalanine and jasmonic acid and application with biofertilizer resulted significant increased on growth, dry yield and quality of Roselle calyces. Treat Phe+JA+Bio produced the significant increase in all biochemical constituents content on dry calyces. This treat could thus be used to enhance the growth and quality of this medicinal plant.

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