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Effect of bio-inoculation and variety in phenolic compounds content of rosella *hibiscus sabdariffa* L. Sepals

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Abstract
The experiment was conducted in Al-Mahaweel district (25 km north of Babil Governorate) during the spring season 2020, for the purpose of knowing the effect of bio-fertilizers on the content of some phenolic substances in the sepals of Rosella, A factorial experiment was applied according to the Complete Randomized Blocks Design (R.C.B.D). The study included two factors, the first factor included three types of gujarat (red, white and lined) and the second factor was biofertilization (adding soil to the soil immediately before planting) at three levels (control, Azotobacter and fungal biofertilization using Mycorrhiza fungi). The results of the statistical analysis showed the red variety was significantly superior to the other two varieties in all phenolic substances, as well as the bio-fungal fertilization treatment achieved the highest means, significantly superior to the rest of the other treatments. The interaction treatment (red variety + Mycorrhiza) recorded the highest means of Sabdaretine(0.099) mg.L^{-1}, Gossypetine(0.183) mg.L^{-1}, Delphinidin-3- glucoside(0.122) mg.L^{-1}, Quercetin(0.120) mg.L^{-1}, Kaempferol(0.143) mg.L^{-1}.

Keywords: Rosella, Bio-Inoculation, phenolic compounds

Introduction
Rosella plant *Hibiscus sabdariffa* L. is one of the medically important plants that belongs to the family Malvaceae. It is native to the hot tropical and subtropical regions, especially in the regions of West Africa, from which it spread to the rest of the world [1].
Rosella contains many medically effective compounds, including phenols, which are chemically defined as compounds consisting of an aromatic ring carrying one or more hydroxyl groups. From a botanical point of view, they are secondary metabolic products formed in the plant in different quantities depending on the characteristics of the distinctive plant products of each plant type, which it depends directly on the quantity and quality of available phenols. These compounds have important biological effects that differ according to the plant. They have been used for pharmaceutical use to be a useful medicinal substance for humans [2]. These compounds have aroused the interest of many researchers and pharmacists and have been introduced in several vital fields. They have been used as antioxidants for viruses and microbes, and they have the ability to increase the body's immunity against pathogenic germs [3]. Phenolic compounds are concentrated in the sepals of the Gujarat plant, and the most important of these compounds are (Flavonoids, Riboflavin, (hibiscetin 3-glucoside), Hibiscitrin, sabdaritrin, gossypitrin, gossytrin and other gossypetin glucosides**,** chlorogenic acid, protocatechuic acid, pelargonidic acid, eugenol, quercetin, luteolin and the sterols -sitosterol and ergosterol [4].
The use of bio-fertilizers is one of the modern technologies that have been used to reduce or reduce the use of chemical fertilizers, which plays an important role in reducing environmental pollution, maintaining biological balance, improving soil properties, absorbing water and nutrients by roots, and increasing soil organic matter content as a result of its physiological ability to analyze organic matter and thus increase the readiness of Metallic Elements [5, 6]. It also plays an important role in promoting plant growth through its ability to fix atmospheric nitrogen and increase the readiness of phosphorous, potassium and other elements. It is characterized by its ability to secrete some growth hormones important for plant growth and development such as gibberellins, auxins and cytokinins [7, 8].

Based on the above-mentioned, this study aimed to find out the effect of bio-fertilizers on the content of some phenolic substances in the sepals leaves.

Materials and Methods

The experiment was conducted in Al-Mahaweel district (25 km north of Babil Governorate) during the spring season 2020, for the purpose of knowing the effect of bio-fertilizers on the content of some phenolic substances in the sepals of Rosella, The seeds of three varieties of Rosella were obtained from the Gujarat Tea Development Project in Al-Diwaniyah of the Al-Diwaniyah Agriculture Directorate. The experimental land was plowed by two orthogonal plows, then it was smoothed and leveled, and then divided into three replication, each replicate included 27 experimental units with an area (3x4) m^2. Triple super phosphate in the form of 160 $kg.ha^{-1}$ in one batch before planting, then adding 100 kg.ha-1 nitrogen fertilizer in the form of urea added in two stage, the first after thinning and the second before flowering [9]. The seeds were planted on (1/4/2016), where the seeds were planted in a hollow in the upper third of the meadow, the distance between one hole and another is 50 cm, alternately on both sides of the meadow, with 3 seeds per hole, at a depth of 3-5 cm, then the field was given Irrigation of cultivation without immersion until germination is completed and when it reaches a height of [10-15] cm, the thinning and patching operations were carried out, after which the plants were watered as needed, with all crop service operations such as weeding, weeding and other control. The study included two factors, the first factor included three types of gujarat (red, white and lined) and the second factor was biofertilization (adding soil to the soil immediately before planting) at three levels (control, Azotobacter and fungal biofertilization using Mycorrhiza fungi)**.** A factorial experiment was applied according to the Complete Randomized Blocks Design (R.C.B.D). The means were compared according to the Least Significant Desgin (L.S.D) test under the probability level of 0.05 using the statistical program Genstat [10].

The content of the sepals leaves of the gujarat plant was estimated from the active substances using a high performance liquid chromatography (HPLC) device. The solution was placed in an ultrasonic device at a temperature of 50 °C for a period of 30 minutes. The resulting solution was filtered with a filter paper, then the filter was placed in the rotary evaporator and at a temperature less than 50 °C until dryness, then 1 ml of Acetonitile was added in three batches and the solution was filtered by means of a 0.45 ml microfilter. Micron, the conditions and stability of the device were controlled, then 20 mm of the sample was injected into a high-performance chromatograph (HPLC) of type (LC-10A) manufactured by Shimadzu Japanese company, and the estimation of the active substances was carried out in the Materials Research Department / Ministry of Science and Technology. As the process of separating the compounds and determining their quality and quantity compared to the standard materials on the separation column and under the same conditions.

Table 4: The retention time and bundle area of the active substances in the sepals of the Rosella plant

Seq	Subject	Retention Time	Area (Movlt)
1	Sabdaretine	3.976	11121824
2	Hibiscetine	4.626	39817950
3	Gossypetine	6.035	1965411
4	Delphinidin-3- glucoside	7.007	31312350
5	Quercetin	3.846	8035000
6	Kaempferol	6.329	1795016

Results

The Results of Table (2) indicate that there are significant differences between the varieties in the content of their sepals from Sabdaretine, as the red variety recorded the highest mean of (0.098) $mg.L^{-1}$, while the white variety achieved the lowest mean of (0.076) $mg.L^{-1}$.

The addition of bio- fertilizer led to a significant increase in the content of Sabdaretine sepals. The treatment (Mycorrhiza) achieved the highest mean of (0.093) $mg.L^{-1}$, while the control treatment recorded the lowest mean of (0.085) $mg.L^{-1}$.

The interaction between the variety and the bio- fertilizer led to significant differences, as the interaction (red variety + Mycorrhiza) achieved the highest mean of (0.099) $mg.L^{-1}$, while the interaction (Lined variety + control) gave the lowest mean of (0.082) $mg.L^{-1}$.

Table 2: Effect of biofertilization and Variety In content of Rosella Sepals from Sabdaretine ($mg.L^{-1}$)

Biofertilizer / Variety	0	Azotobacter	Mycorrhiza	Mean
Red	0.094	0.097	0.099	0.098
White	0.084	0.089	0.087	0.076
lined	0.082	0.092	0.085	0.082
Mean	0.085	0.089	0.093	
Interaction=0.31	$L.S.D_{(0.05)}$	Variety=0.15	biofertilizer=0.15	

The Results of Table (3) indicate that there are significant differences between the varieties in the content of their sepals from Hibiscetine, as the red variety recorded the highest mean of (0.335) $mg.L^{-1}$, while the white variety achieved the lowest mean of (0.233) $mg.L^{-1}$.

The addition of bio- fertilizer led to a significant increase in the content of Hibiscetine sepals. The treatment (Mycorrhiza) achieved the highest mean of (0.715) $mg.L^{-1}$, while the control treatment recorded the lowest mean of (0.299) $mg.L^{-1}$.

The interaction between the variety and the bio- fertilizer led to significant differences, as the interaction (red variety + Azotobacter) achieved the highest mean of (0.729) $mg.L^{-1}$, while the interaction (Lined variety + control) gave the lowest mean of (0.486) $mg.L^{-1}$.

Table 3: Effect of biofertilization and Variety In content of Rosella Sepals from Hibiscetine ($mg.L^{-1}$)

Biofertilizer / Variety	0	Azotobacter	Mycorrhiza	Mean
Red	0.503	0.729	0.522	0.335
White	0.486	0.694	0.471	0.233
lined	0.497	0.723	0.516	0.329
Mean	0.299	0.495	0.715	
$L.S.D_{(0.05)}$	Variety=0.21	biofertilizer=0.21	Interaction=0.42	

The Results of Table (4) indicate that there are significant differences between the varieties in the content of their sepals from Gossypetine, as the red variety recorded the highest mean of (0.135) mg.L^{-1}, while the white variety achieved the lowest mean of (0.125) mg.L^{-1}.
The addition of bio- fertilizer led to a significant increase in the content of Gossypetine sepals. The treatment (Mycorrhiza) achieved the highest mean of (0.174) mg.L^{-1}, while the control treatment recorded the lowest mean of (0.161) mg.L^{-1}.
The interaction between the variety and the bio- fertilizer led to significant differences, as the interaction (red variety + Mycorrhiza) achieved the highest mean of (0.183) mg.L^{-1}, while the interaction (Lined variety + control) gave the lowest mean of (0.151) mg.L^{-1}.

Table 4: Effect of biofertilization and variety in content of rosella sepals from gossypetine (mg.L^{-1})

Biofertilizer / Variety	0	Azotobacter	Mycorrhiza	Mean
Red	0.165	0.180	0.183	0.135
White	0.151	0.167	0.160	0.125
lined	0.160	0.176	0.173	0.130
Mean	0.161	0.171	0.174	
L.S.D$_{(0.05)}$	Variety=0.12	biofertilizer=0.12	Interaction=0.25	

The Results of Table (5) indicate that there are significant differences between the varieties in the content of their sepals from Delphinidin-3- glucoside, as the red variety recorded the highest mean of (0.058) mg.L^{-1}, while the white variety achieved the lowest mean of (0.025) mg.L^{-1}.
The addition of bio- fertilizer led to a significant increase in the content of Delphinidin-3- glucoside sepals. The treatment (Mycorrhiza) achieved the highest mean of (0.120) mg.L^{-1}, while the control treatment recorded the lowest mean of (0.099) mg.L^{-1}.
The interaction between the variety and the bio- fertilizer led to significant differences, as the interaction (red variety + Mycorrhiza) achieved the highest mean of (0.122) mg.L^{-1}, while the interaction (Lined variety + control) gave the lowest mean of (0.085) mg.L^{-1}.

Table 5: Effect of biofertilization and variety in content of rosella sepals from delphinidin-3- glucoside (mg.L^{-1})

Biofertilizer / Variety	0	Azotobacter	Mycorrhiza	Mean
Red	0.103	0.130	0.122	0.058
White	0.085	0.119	0.121	0.025
lined	0.096	0.127	0.118	0.042
Mean	0.099	0.116	0.120	
L.S.D$_{(0.05)}$	Variety=0.25	biofertilizer=0.25	Interaction=0.32	

The Results of Table (6) indicate that there are significant differences between the varieties in the content of their sepals from Quercetin, as the red variety recorded the highest mean of (0.053) mg.L^{-1}, while the white variety achieved the lowest mean of (0.020) mg.L^{-1}.
The addition of bio- fertilizer led to a significant increase in the content of Quercetin sepals. The treatment (Mycorrhiza) achieved the highest mean of (0.135) mg.L^{-1}, while the control treatment recorded the lowest mean of (0.071) mg.L^{-1}.
The interaction between the variety and the bio- fertilizer led to significant differences, as the interaction (red variety + Mycorrhiza) achieved the highest mean of (0.120) mg.L^{-1}, while the interaction (Lined variety + control) gave the lowest mean of (0.055) mg.L^{-1}.

Table 6: Effect of biofertilization and Variety In content of Rosella leaves from Quercetin (mg.L^{-1})

Biofertilizer / Variety	0	Azotobacter	Mycorrhiza	Mean
Red	0.071	0.079	0.120	0.053
White	0.195	0.063	0.071	0.020
lined	0.055	0.071	0.061	0.033
Mean	0.071	0.084	0.135	
L.S.D$_{(0.05)}$	Variety=0.066	biofertilizer=0.066	Interaction=0.013	

The Results of Table [7] indicate that there are significant differences between the varieties in the content of their sepals from Kaempferol, as the red variety recorded the highest mean of (0.133) mg.L^{-1}, while the white variety achieved the lowest mean of (0.118) mg.L^{-1}.
The addition of bio- fertilizer led to a significant increase in the content of Kaempferol sepals. The treatment (Mycorrhiza) achieved the highest mean of (0.093) mg.L^{-1}, while the control treatment recorded the lowest mean of (0.085) mg.L^{-1}.
The interaction between the variety and the bio- fertilizer led to significant differences, as the interaction (red variety + Mycorrhiza) achieved the highest mean of (0.143) mg.L^{-1}, while the interaction (Lined variety + control) gave the lowest mean of (0.126) mg.L^{-1}.

Table 7: Effect of bio-fertilization and variety in content of rosella sepals from kaempferol (mg.L^{-1})

Biofertilizer / Variety	0	*Azotobacter*	*Mycorrhiza*	Mean
Red	0.141	0.149	0.141	0.133
White	0.128	0.137	0.129	0.118
lined	0.135	0.142	0.136	0.128
Mean	0.126	0.135	0.143	
L.S.D$_{(0.05)}$	Variety=0.01	biofertilizer=0.01	Interaction=0.02	

Discussion

The reason for the difference in the phenol content of the Rosella varieties in their sepals may be due to the different nature and genetic factor and their suitability to the environmental conditions of the region. These results are in agreement with the findings [11, 12, 13, 14].

The increase in the content of the sepals of phenols as a result of adding bio-fertilizer may be due to the positive role of bio-fertilizer in increasing the readiness of the nutrients necessary for the plant and then their absorption, transport and accumulation within the plant tissues in full concentrations, especially phosphorous. These results are in line with what was found(ز The positive role of bio-fertilizer in increasing the readiness of the nutrients necessary for the plant and then their absorption, transport and accumulation within the plant tissues in full concentrations, especially phosphorous. These results are in line with what was found. [15, 16, 17].

Conclusion

In light of the obtained results, it can be concluded that the red variety is the best in the content of the sepals of phenols, and the addition of the fungal bio-vaccine achieved the best results.

References

1. Abbas MK, Ali AS. Effect of foliar application of NPK on some growth characters of two cultivars of roselle (*Hibiscus sabdariffa* L.). American Journal of Plant Physiology. 2011;6(4):220-227.
2. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. Journal of chromatography A. 2004;1054(1-2):95-111.
3. Umar Lule S, Xia W. Food phenolics, pros and cons: A review. Food Reviews International. 2005;21(4):367-388.
4. Baxter K, Driver S, Williamson E. (Eds.). Stockley's herbal medicines interactions. London, UK: Pharmaceutical Press; c2013.
5. Sharma AK. Bio-fertilizers for Sustainable Agriculture. A Handbook of Organic farming Agrobios, India, 2002, 17-18.
6. Christian KR, Jackson JC. Changes in total phenolic and monomeric anthocyanin composition and antioxidant activity of three varieties of sorrel (*Hibiscus sabdariffa*) during maturity. Journal of food composition and analysis. 2009;22(7-8):663-667.
7. Vessey JK. Plant growth promoting rhizobacteria as bio-fertilizer. Plant and Soil. 2003;255(2):571-586.
8. Meraiyebu AB, Olaniyan OT, Eneze C, Anjorin YD, Dare JB. Anti-inflammatory activity of methanolic extract of *Hibiscus sabdariffa* on carrageenan induced inflammation in wistar rat. International Journal of Pharmaceutical Science Invention. 2013;2(3):22-24.
9. Nasrullah Y. Medicinal plants. Baghdad University. faculty of Agriculture. Ministry of Higher Education and Scientific Research. The Republic of Iraq; c2012.
10. Al-Rawi KM, Khalafallah AAM. Design and analysis of agricultural experiments (University of Dar Al Kutub for printing and publishing; c1980.
11. Hassoon AS, Ussain MH, Harby HH. Effect of spraying of humic acid on sepals extract content from some antioxidants for three varieties of rosella (*Hibiscus sabdariffa* L.). Plant Archives. 2018;18(1):1129-1133.
12. Hassoon AS, Alnuaimi JJJ. The Effect of Biofertilization in Fatty Acids Content for Many Flax Varieties. In IOP Conference Series: Earth and Environmental Science. November 2021;910(1):012042. IOP Publishing.
13. Hussain MH, Hassoon AS. Measurement of some medical substances in rosella plant (*Hibiscus Sabdariffa* L.) extract using hplc technique under the influence of different treatments. Plant Archives. 2018;18(2):2469-2476.
14. Hasson AS, Ramadan EL, Hussain MH. Effect of salicylic acid and seaweed extract in the content of sepals of some active medical compounds for several varieties of roselle Hibiscus Sabdariffa L. Int J STEM Educ. 2017;4(4):7068-73.
15. Alnuaimi JJJ, Hassoon AS, Almyali AAH. Evaluation of the performance of four genotypes of Corn (*Zea mays* L.) and path coefficient analysis by Bacterial biofertilizers effects. Eco. Env. & Cons. 2019;26(1):262-270.
16. Hussain MH, Jader JJ, Hassoon AS. Effect of Bio-Fertilization and Foliar Spraying in the Mustard Seed Content Brassica Alba L. from Some Fatty Acids. Indian Journal of Public Health Research & Development. 2020;11(4).
17. Hassoon AS, Kadhim AA, Al-Taee MM. Role of Fungal Biofertilizers in Agricultural Production. J Agric Sci Crop Res. 2020;1(1):103.