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EFFECT OF SPRAYING OF HUMIC ACID ON SEPALS EXTRACT CONTENT FROM SOME ANTIOXIDANTS FOR THREE VARIETIES OF ROSELLA (*HIBISCUS SABDARIFFA* L.)

Ali S. Hassoon¹, Madeha H. Ussain² and Huda H. Harby³

¹Plant Production Techniques, Al-Musaib Tech. College, Al-Furat Al-Awsat Tech. University, Iraq.

²Department of Pharmacy, Medical Institute Tech. Mansour, Middle Tech. University, Iraq.

³Plant Production Techniques, Agriculture College, Al-Muthanna University, Iraq.

Abstract

The experiment was carried out in the fields of one of the farmers of the Musaib area of Babil Governorate during the summer season 2017 to study the effect of Spraying of Humic Acid on Sepals Extract Content From Some Antioxidants for Three Varieties of Rosella. Experimental factorial is applied according to complete randomized blocks design (R.C.B.D). Included the first factor three varieties (red, lined and white). The second factor is Humic acid in three Concentrations (2, 4, and 6) mg/lit In addition to the control treatment. The results showed that the varieties and Humic and their interaction affected all measured substances (Apigenin, Lutelion, Chrysin, Myricetin, Hesperetin, Genistein), red variety dominated on other varieties, also Spray treatment by Humic acid 6 mg/lit And their Interaction dominated in All measured substances.)

Key words : Rosella, varieties, humic acid, antioxidants.

Introduction

The medical plants are of great importance in agricultural, industrial and medical products, as they are the main source of pharmaceuticals and active substances in the pharmaceutical industry (Mahmoud, 2008) and are characterized by being more beneficial and safe in treatment and less expensive compared to manufactured chemical pharmaceutical (Borokini and Omotayo, 2012). Among these plants is the Rosella (*Hibiscus sabdariffa* L.), which is one of the important summer medicinal plants belonging to the Malvaceae family. The tropical and subtropical regions of the African continent are its original home (Toukara *et al.*, 2011), while in the Arab world it is cultivated in Saudi Arabia, Egypt and Sudan (Eslaminejad and Zakaria, 2011). Rosella is used medicinally to treat high blood pressure, atherosclerosis, anti-oxidation, lowering cholesterol, analgesic, colds, antifungal, microbes, ulcers and others (Kiliç *et al.*, 2011). In addition to its many food and industrial uses, it is used as a refreshing drink, colored and tasteful, the manufacture of jams, ice cream, and sweets, and is used in the manufacture of cosmetics and soaps (Saadi, 2006).

The productivity of this plant and its medical compounds content can be increased by using several methods, including spraying organic fertilizer as a modern agricultural processes that lead to a healthy environment free from the risks of pollution of soil and humans directly or indirectly compared to chemical fertilizer, Humic acid is an important organic nutrient consisting of organic acids (humic and folic acid), amino acids and nutrients resulting from the decomposition of organic matter and is used for increasing production and quality. It also contains a proportion of the major nutrients, especially NPK and carbon and the varying amounts of microelements (Moghadam *et al.*, 2014). It was found (Madiha *et al.*, 2014) that the spraying of organic fertilizer has caused a significant increase in the content of the sepals of the medical compounds of the Rosella and found (AlTohafi *et al.*, 2015) that sprayed the acid of the humic has improved the qualities of growth and yield of the Rosella. Due to the medicinal importance of this plant, the study aimed to investigate the effect of spraying of humic acid in the content of the sepals extract from some active substances and to three varieties of Rosella.

Table 1 : Physical and chemical properties of soil.

Character	pH	Ec	N Mg.kg ⁻¹	P Mg.kg ⁻¹	K Mg.kg ⁻¹	Organic Matter (%)	Soil separators			Texture
							Sand gm.kg ⁻¹ soil	Loamy gm.kg ⁻¹ soil	Clay gm.kg ⁻¹ soil	
Value	7.5	2.4	35	14.28	270	3.2	127	552	315	Sand loamy

Table 2 : The Retention Time and the area of some antioxidants in the sepals of the Rosella.

Area	Retention Time	Subject	Seq
502131	4.360	Apigenin	1
1136933	4.709	Lutelion	2
1428763	5.109	Chrysin	3
1753526	6.987	Myricetin	4
525162	8.540	Hesperetin	5
726734	9.230	Genistein	6

Table 3 : Chromatographic Separation conditions for Some antioxidants in the sepals using (HPLC)

Type (C-18), regeneration (250 * 4.6 mm Id), syringe size 5µm	Column
70% CH ₃ OH:30% Tetra Hydro Furan (THF)	M.Phase
1 ml/min	F.Rate
Uv-vis at 338 nm	Detector
1) 20 µm	Volume of injection

Materials and Methods

The experiment was carried out in the fields of one of the farmers of the Musaiab area of Babil Governorate during the summer season 2017 to study the effect of Spraying of Humic Acid on Sepals Extract Content From Some Antioxidants for three varieties of Rosella. Experimental factorial is applied according to complete randomized blocks design (R.C.B.D). Included the first factor three varieties (red, lined and white). The second factor is Humic acid in three Concentrations (2, 4 and 6) mg/lit In addition to the control treatment. Five sprays between spray and another 20 days. And the land of the experiment with its plowing two planks orthogonal and Smoothing, and was settled, then divided the field of an experiment to three blocks. Each block included 12 experimental units with an area of 3×4 m². The experimental unit included 4 lines with a length of 3 meters and the distance between the line and the last 75 cm. The seed was grown on 15/3/2017. And random samples were taken from the soil of the field before planting for the seasons in depth (0-30 cm) to know the properties of physical and chemical soil. Soil samples were analyzed

in the Soil Department laboratories - Musaiab Technical Institute - Al-Furat Al-Awsat Technical University as shown in table 1 the soil.

Then all the service operations were done from irrigation, fertilization, lightening, weeding, weeding and fighting. At the harvest, 5 random plants were selected for each of the intermediate lines from which the measurements were taken, and the outer lines were left as guard lines. The content of the sepals of some of the antioxidants was estimated by taking the sepals and drying the air. The active substances (Apigenin, Lutelion, Chrysin, Myricetin, Hesperetin, Genistein), were extracted according to the method he mentioned (Obouayeba *et al.*, 2014) and measured the content of the oxidative leaves of the antioxidants through the duration of their retention by HPLC (High-Performance Liquid Chromatography), Active compounds in Materials Research Department, Ministry of Science and Technology. The following equation calculated the concentration of the separated materials in the sample:

$$\text{Sample conc.} = \frac{\text{Sample area}}{\text{Standard solution area}} \times \text{Dilution number} \times \text{Standard solution conc.}$$

Results

Apigenin

The results of table 4 shown that the superiority of the red variety of the other varieties in the sepals content of Apigenin, which gave the highest mean of 9.82 mg/l, while the white variety gave the lowest mean of 6.69 mg /l. The results showed that the treatment of Humic 6 mL / L gave higher mean of 12.20 mg / L. While the control treatment gave the lowest mean of 3.73 mg / L as for the interaction between the variety and the humic acid. The results showed that the combination (Red + humic acid at 6 ml / L), gave the highest mean of Apigenin in the sepals (14.22) mg/l, while the combination (White+control treatment) presented the lowest mean (3.21 mg/l).

Lutelion

The results of table 5 show that the superiority of the red variety of the other varieties in the content of the sepals of the Lutelion and gave the highest mean of 9.82

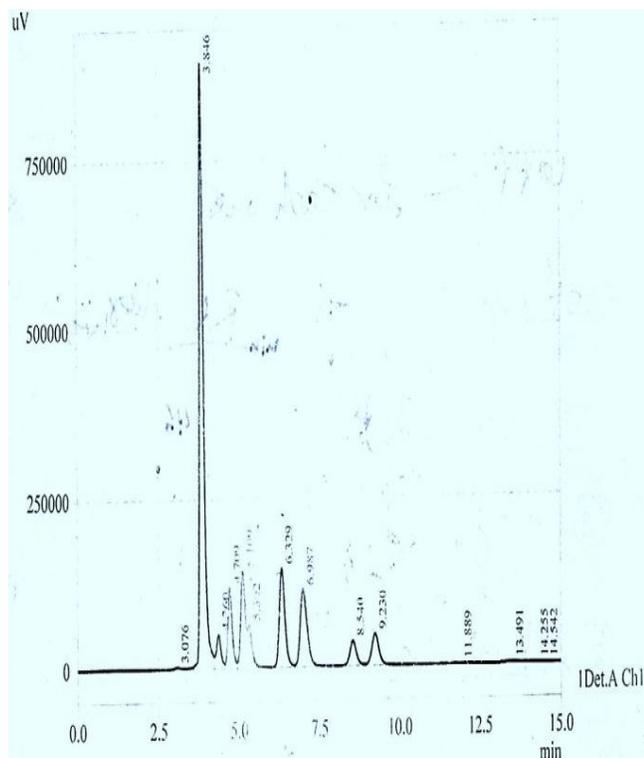


Fig. 1 : The standard model curve of the sepals extract.

Chrysin

The results of table 6 shown that the superiority of the red variety of the other variations in the content of the sepals of the Chrysin and gave the highest mean of (5.29 mg/l), while the white variety gave the lowest mean of 3.60 mg/l. The results showed that the treatment of Humic 6 mL/L gave a higher mean of 7.94 mg/L. While the control treatment gave the lowest mean of 1.52mg/L as for the interaction between the variety and the humic acid. The results showed that the combination (Red + humic acid at 6 ml / L), gave the highest mean of Chrysin in the sepals (8.89) mg / l, while the combination (White +control treatment) presented the lowest mean (1.18 mg/ l).

Myricetin

The results of table 7 shown that the superiority of the red variety of the other variations in the content of the sepals of the Myricetin and gave the highest mean of 4.08 mg/l, while the white variety gave the lowest mean of 2.10 mg/l. The results showed that the treatment of Humic 6 mL / L gave a higher mean of 4.83 mg/L, while the control treatment gave the lowest mean of 1.36 mg/

Table 4 : Effect of Humic acid in the Sepals content of Apigenin (mg / l) for three varieties of Rosella.

Mean varieties	ml/16	ml/14	ml/12	0	Humic Varieties	
					Red	Lined
9.82	14.22	13.06	7.80	4.22		
7.33	11.46	7.25	6.88	3.76		
6.69	10.92	6.64	6.02	3.21		
	12.20	8.98	6.90	3.73		Mean Humic

Table 5 : Effect of Humic acid in the Sepals content of Lutelion (mg / l) for three varieties of Rosella.

Mean varieties	ml/16	ml/14	ml/12	0	Humic Varieties	
					Red	Lined
5.45	9.27	6.47	4.03	2.05		
4.77	8.79	5.96	2.71	1.64		
3.53	6.71	4.15	2.13	1.16		
	8.25	5.52	2.95	1.61		Mean Humic

mg/l, while the white variety gave the lowest mean of 8.25 mg / l. The results showed that the treatment of Humic 6 mL / L gave higher mean of 12.20 mg / L. While the control treatment gave the lowest mean of 1.61mg/ L as for the interaction between the variety and the humic acid. The results showed that the combination (Red + humic acid at 6 ml / L), gave the highest mean of Lutelion in the sepals (9.27) mg / l, while the combination (White +control treatment) presented the lowest mean (1.16 mg / l).

L as for the interaction between the variety and the humic acid. The results showed that the combination (Red + humic acid at 6 ml / L), gave the highest mean of Myricetin in the sepals (6.43 mg/l), while the combination (White +control treatment) presented the lowest mean (1.30 mg/ l).

Hesperetin

The results of Table (8) shown that the superiority of the red variety of the other variations in the content of the sepals of the Hesperetin and gave the highest mean of 4.53 mg / l, while the white variety gave the lowest

Table 6 : Effect of Humic acid in the Sepals content of Chrysin (mg / l) for three varieties of Rosella.

Mean varieties	ml/16	ml/14	ml/12	0	Humic Varieties
5.29	8.89	6.06	4.18	2.03	Red
4.87	8.51	5.43	2.11	1.36	Lined
3.60	6.42	4.96	1.87	1.18	White
	7.94	5.48	2.72	1.52	Mean Humic

Table 7 : Effect of Humic acid in the Sepals content of Myricetin (mg / l) for three varieties of Rosella.

Mean varieties	ml/16	ml/14	ml/12	0	Humic Varieties
4.08	6.43	2.75	1.63	1.45	Red
2.19	4.08	1.76	1.60	1.33	Lined
2.10	3.98	1.65	1.48	1.30	White
	4.83	2.05	1.57	1.36	Mean Humic

Table 8 : Effect of Humic acid in the Sepals content of Hesperetin (mg / l) for three varieties of Rosella.

Mean varieties	ml/16	ml/14	ml/12	0	Humic Varieties
4.53	6.27	6.06	3.51	2.29	Red
3.08	4.96	3.92	1.88	1.57	Lined
2.50	4.29	2.81	1.57	1.35	White
	5.17	4.26	2.32	1.73	Mean Humic

Table 9 : Effect of Humic acid in the Sepals content of Genistein (mg / l) for varieties of Rosella.

Mean varieties	ml/16	ml/14	ml/12	0	Humic Varieties
4.06	7.27	4.41	2.96	1.61	Red
2.56	4.32	2.95	1.73	1.25	Lined
2.14	4.03	2.16	1.38	1.02	White
	5.20	3.17	2.02	1.29	Mean Humic

mean of 2.50 mg / l. The results showed that the treatment of Humic 6 mL / L gave higher mean of 5.17mg/L, while the control treatment gave the lowest mean of 1.73 mg / L as for the interaction between the variety and the humic acid. The results showed that the combination (Red + humic acid at 6 ml / L), gave the highest mean of Hesperetin in the sepals (6.27) mg / l, while the combination (White +control treatment) presented the lowest mean (1.35 mg / l).

Genistein

The results of table 9 shown that the superiority of the red variety of the other variations in the content of the sepals of the Genistein and gave the highest mean of 4.06 mg/l, while the white variety gave the lowest mean of 2.14 mg / l. The results showed that the treatment of Humic 6 mL/L gave higher mean of 5.20 mg/L, while the control treatment gave the lowest mean of 1.92 mg/L as for the interaction between the variety and the humic acid. The results showed that the combination (Red +

humic acid at 6 ml / L), gave the highest mean of Genistein in the sepals (7.27 mg / l), while the combination (White +control treatment) presented the lowest mean (1.02 mg / l).

Discussion

The results shown in the tables (4, 5, 6, 7, 8, 9) indicate that the red variety exceeds on the rest of the variations in all the measured materials. This may be due to the difference of genetic factors among the varieties. Due to the suitability of the variety to the environmental conditions and the soil of the region (table 3). This corresponds to what they reached (Majeed and Ali, 2011) the difference between the two types of red and white variety in the content of the leaves of the active substances. The increase in the content of the sepals of the antioxidants by increasing the spray of humic acid, especially the concentration of 6 ml / liter may be due to the role of the acid in the provision of nutrients and ready to increase the efficiency of absorption by the plant and

thus increase the process of photosynthesis and the representation of carbohydrates that reflect positively on the process of storage vehicles. In addition to the role of acid in increasing the readiness of the major and minor nutrients and the effect of this increase in growth by about one year and improve the enzymatic performance and metabolic processes in particular and the reflection on the increase of active substances (Hendawy, 2008). These results agreed with the findings of Nasser, (2010) that there is an increase in the active substances in the leaves of the rosella treated with humic acid.

Conclusion

From the results, it was observed that the red variety is superiority on the other variations in the sepals of the antioxidants, it also gave the treatment of spraying if a humic acid (6 ml/l) and their interactions best results.

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