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Physiological effects of henna aqueous extract on growth, yield, active constituent and anatomical structure of roselle (*Hibiscus sabdariffa* L.) plant

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Abstract

This research was conducted at the Experimental Farm of Medicinal and Aromatic Plants Research Department in El-Kanater El-Khairia, during the two successive seasons 2019 and 2020. The aim of this study is to investigate the effect of spraying roselle plants with henna aqueous extract at concentrations (10, 20, 30 and 40 g/l) on growth, yield, calyces quality (anthocyanin content and acidity %) and anatomical structure. In general, the results indicated that all doses of the henna aqueous extract significantly increased the growth, yield and calyces quality as compared with control in the two seasons. This increment was parallel to the gradual increase in the dose of henna aqueous extract from 0 up to 30 g/l. In stem anatomical studies, henna extract at 30 g/l also recorded the highest results for all characters. The anatomical structure of the leaf followed approximately the same direction in the stem.

Keywords: Roselle plants, henna aqueous extract, anthocyanin, acidity, anatomical structure

Introduction

Roselle (*Hibiscus sabdariffa* L.) belongs to family Malvaceae. Roselle is cultivated mainly for its calyces. It has an economic importance due to its medicinal uses. Pharmacological studies mentioned that calyces of roselle have numerous effects including antibacterial, antidepressant, cardiac and antioxidant [1]. Roselle calyces are rich in anthocyanins, the major pigment reported as delphinidin-3-sambubioside (known as delphinidin-3-xylosylglucoside or hibiscine) and cyanidin-3-sambubioside (known as cyanidin-3-xylosylglucoside or gossypicyanin). Also, they have high amounts of organic acids, such as citric acid, malic acid, tartaric acid, and ascorbic acid [2]. The dried calyces can be soaked in water to prepare a cold drink or may be boiled in water and taken as a hot drink. The calyces are surrounding the fruits (capsules). Roselle seeds are good sources of lipid-soluble antioxidants and γ -tocopherol. Also, seeds are rich in minerals such as potassium, magnesium calcium, and phosphorus [3].

Henna (*Lawsonia inermis* L.) is an important medicinal plant, belonging to the family Lythraceae. Numerous studies revealed that the henna plant has biological activities such as antimicrobial, anti-inflammatory, anticancer, antioxidant, and many other activities. In addition to, the dried leaves of henna is an important cosmetic dye. The pharmacological analysis of henna extract reported that the presence of phenolic, flavonoids, carbohydrates, alkaloids, tannins, protein, quinines, and coumarins. The major active constituent in henna is 2-hydroxy-1-4 naphthoquinone [4].

This work aimed to investigate the effect of henna aqueous extract on increasing growth, yield, some chemical components and anatomical features of roselle plant.

Materials and Methods

A field experiment was conducted during the two seasons of 2019 and 2020 at the Experimental Farm of Medicinal and Aromatic Plants Research Department in El-Kanater El-Khairia.

Seeds of roselle were obtained from the Experimental Farm of Medicinal and Aromatic Plants Research Department. Seeds were sown on April 20th at a distance of 60 x 50 cm in the two seasons. Four seeds were sown per hill; the seedlings were thinned to one plant per hill.

Chemical fertilizers were applied at the recommended level in three doses, using calcium superphosphate (15.5 % P₂O₅), ammonium sulphate (20.6% N), and potassium sulphate (48% K₂O).

The first dose included all phosphorous amounts that were added during soil preparation, the rest (NK) were done in two equal doses, the first was applied 60 days after sowing and the second dose was conducted 45 days after the first one. Henna leaves were obtained from the Experimental Farm of Medicinal and Aromatic Plants Research Department and dried leaves were ground. A 100 g were weighed and put in a liter, which was left to soak for 24 hours, then were filtered and the final volume of extract was restored to one liter, 10 ml of filtrate were dried and used for the determination of phytohormones, lawsone (2-hydroxy-1-4 naphthoquinone), total phenols, flavonoids and alkaloids. Dilutions were prepared (10, 20, 30 and 40 g / l).

The plants were sprayed twice with henna aqueous extract at concentrations of 10, 20, 30 and 40 g/l. The first spray was conducted eight weeks after sowing and the second one was done two weeks thereafter. Identification and quantification of phytohormones; gibberellic acid (GA₃), indole acetic acid (IAA), and abscisic acid (ABA) were carried out using high - performance liquid chromatography (Hewlett Packard, HP1050 liquid chromatography following by the methodology of [5]).

The growth hormones were identified on the basis of the retention time of the standard and the concentration of each hormone was calculated on the basis of peak area.

The quantification of 2-hydroxy-1-4 naphthoquinone (lawsone) was determined according to the method described by [6]. Total phenols content was determined by using Folin-Ciocalteu assay, as described by [7]. Total flavonoids content was determined by using the method described by [8]. Total alkaloids content was determined as recommended by [9].

Table 1: Some components in henna aqueous extract

Components	Dry weight of henna aqueous extract
GA ₃	0.713 (mg/100g dry weight)
IAA	0.055 (mg/100g dry weight)
ABA	0.172 (mg/100g dry weight)
2 -hydroxy- 1-4 naphthoquinone	0.608 (g / 100 g dry weight)
Total phenols	0.7082 (%)
Total flavonoids	129.538 (mg/100g dry weight)
Total alkaloids	0.040 (g / 100 g dry weight)

The experiment was designed in complete randomized blocks

Table 2: Effect of henna aqueous extract on plant height and number of branches /plant during 2019/ 2020 seasons.

Treatments	Vegetative growth			
	1 st season		2 nd season	
	Plant height(cm)	Number of branches /plant	Plant height(cm)	Number of branches /plant
Control	140.43	6.67	153.93	8.00
Henna extract at 10 g / l	165.99	9.00	175.47	10.00
Henna extract at 20 g / l	177.07	11.33	182.73	12.67
Henna extract at 30 g / l	202.18	15.00	210.55	16.33
Henna extract at 40 g / l	190.33	12.00	202.13	14.67
L.S.D.at 5%	2.88	0.64	4.38	1.50

Yield and its components

It was obvious that aqueous extract of henna had a significant effect on the number of fruits /plant, fresh and air dry weights of calyces /plant, and seeds yield /plant compared to control in both seasons as recorded in Table 3. Increasing the

with five treatments; each treatment was replicated three times. The experimental unit was 2.4 x 3 m and contained 24 plants (6 plants/ row).

Roselle plants were harvested on October 15th in the two seasons, and then data were recorded on plant height, number of branches/plant, number of fruits/plant, fresh and dry weight of calyces/plant and seeds yield /plant.

Anthocyanin content was determined in dried calyces using the method described by [10] and acidity was determined according to the method described by [11].

The means were compared using the least significant difference (L.S.D.) test at 5% level, according to [12].

Anatomical studies

Samples of roselle plants represent the concentrations that gave the lowest and highest positive values in their results compared to the control plants were taken for anatomical studies. Specimens included the main stem at its median internode and the leaf blade which developed on the same internode. Samples were killed and fixed for at least 48 hrs in FAA solution (Formalin, Glacial Acetic Acid, and Ethyl Alcohol 70%). After fixation, materials were washed in 50% ethyl alcohol (International Company for Supp. & Med. Industries, Egypt), dehydrated through a normal butyl alcohol (Biochem Company, Egypt) series and embedded in paraffin wax (Hista-Flex™ Prime, Manufactured in the UK). Transverse sections which were cut on a rotary microtome (Leica Company, German) to a thickness of 15 microns were stained with crystal violet-erythrosin (Oxford Lab Chem, Mumbai, India) dyes before mounting in Canada balsam (24 x 50mm, made in China) and cover slips (XX) attached. Micro-technique procedures were carried out according to [13]. Slides were examined microscopically using a micrometer (Leica, German) eyepiece and an average of five readings for each slide was calculated. Slides were photomicrographed.

Results

Vegetative growth

Data recorded in Table 2 indicated that all concentrations of henna aqueous extract significantly increased plant height and number of branches /plant as compared to control in the two seasons. However, the highest values were obtained from the plants sprayed with aqueous extract at 30 g / l in both seasons

concentration gradually increased yield components up to 30 g / l, the highest values were achieved when roselle plants treated with henna aqueous extract at 30 g / l in the two seasons.

Table 3: Effect of henna aqueous extract on number of fruits, fresh and air dry weights of calyces and seeds yield of roselle plants during 2019/ 2020 seasons.

Treatments	1 st season				2 nd season			
	Number of fruits / plant	Fresh weight of calyces/ plant (g)	Air dry weight of calyces/ plant (g)	Seeds yield / plant (g)	Number of fruits / plant	Fresh weight of calyces / plant (g)	Air dry weight of calyces/ plant (g)	Seeds yield / plant (g)
Control	44.33	165.67	17.08	21.34	52.67	170.17	19.03	25.50
Henna extract at 10 g / l	63.00	191.73	21.35	30.13	76.00	211.50	22.63	35.73
Henna extract at 20 g / l	83.67	220.47	25.10	39.60	90.67	231.17	27.23	41.70
Henna extract at 30 g / l	101.67	267.05	36.34	54.23	120.00	275.27	38.47	56.13
Henna extract at 40 g / l	92.33	248.37	31.41	47.67	105.00	252.37	33.05	48.18
L.S.D.at 5%	7.20	17.96	3.04	5.89	13.18	15.81	3.30	6.09

Chemical composition of calyces

The quality of calyces included anthocyanin content and acidity percentage. Data presented in Table 4 showed that all treatments of the henna aqueous extract significantly increased the quality of calyces compared with control, which

recorded the lowest values in the two seasons. The highest anthocyanin content and acidity percentage in this concern were recorded with aqueous extract at 30 g / l giving, 1.643 and 1.809 anthocyanin (g /100 g dry weight) and acidity percentage (1.570 and 1.630 %) in both seasons.

Table 4: Effect of henna aqueous extract on anthocyanin content and acidity percentage in calyces of roselle plants during 2019/ 2020 seasons.

Treatments	Quality of calyces			
	1 st season		2 nd season	
	Anthocyanin (g / 100 g dry weight)	Acidity % of dry weight	Anthocyanin (g / 100 g dry weight)	Acidity % of dry weight
Control	1.097	0.825	1.120	1.042
Henna extract at 10 g / l	1.210	1.218	1.268	1.230
Henna extract at 20 g / l	1.350	1.313	1.394	1.362
Henna extract at 30 g / l	1.643	1.570	1.809	1.630
Henna extract at 40 g / l	1.482	1.410	1.571	1.510
L.S.D.at 5%	0.089	0.092	0.077	0.073

From above results, it is necessary to confirm that the application of henna aqueous extract at 30 g / l gave the highest growth characters, yield and quality of roselle plants.

Anatomical studies

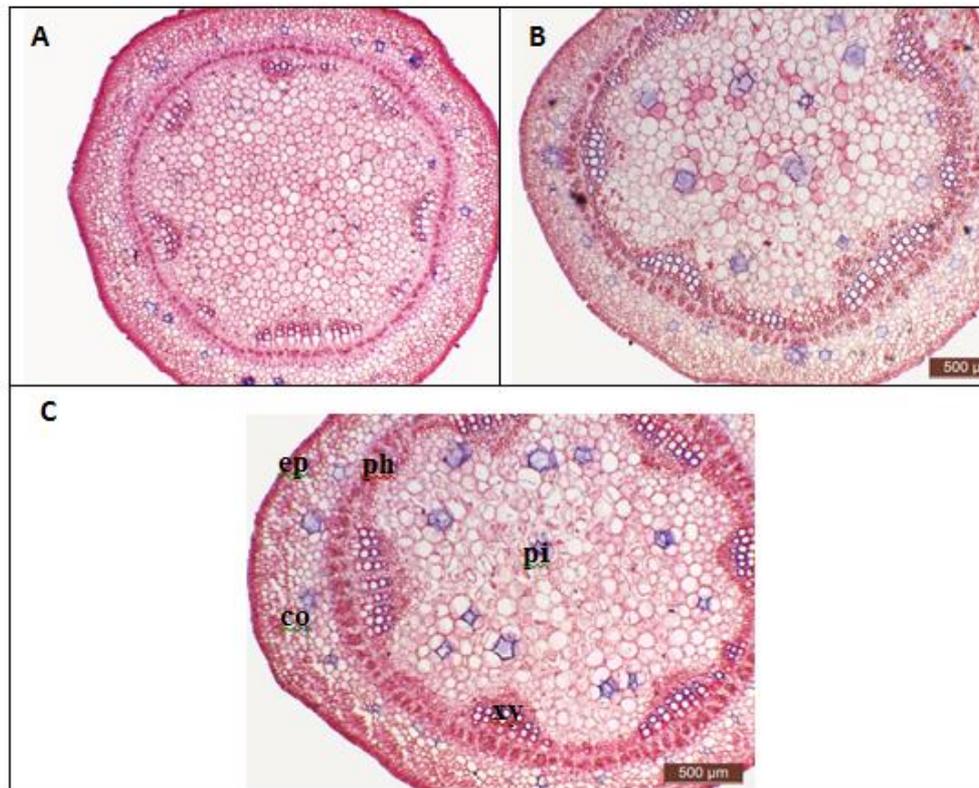
Full microscopic measurements of the transverse sections through the median internode of the main stem and the leaf blade on the same internode of roselle plants treated with henna aqueous extract concentrations at 10 and 30 g/l as well as the control plant are presented in Tables (5 and 6). Microphotographs illustrating these treatments are shown in Figures (1and 2).

The stem anatomical parameters

Data in Table 5 and Figure 1 show that henna aqueous extract at a concentration of 30 g/l recorded the highest stem diameter (3950.1 µm). This increase in stem diameter could be attributed to the increase in the thickness of epidermis, cortex, fiber, phloem tissue, and xylem tissue, as well as the diameter of vessel and pith. The increase percentages were 176.8, 140.9, 331.4, 140.5, 81.0, 56.8, and 53.3 %, respectively, compared to the control plants. While henna extract at a concentration of 10 g/l scored the lowest positive results with increase percentages of 74.4, 56.5, 131.4, 124.9, 59.4, 4.6, and 35.2 %, respectively, compared to the results of the control plants for the previously mentioned characters.

Table 5: Effect of different henna extract concentrations on stem anatomical characters of *Hibiscus sabdariffa* L. plants.

Stem anatomical characters (µm)	Treatments (henna extract)				
	control	10 g/l	±% to cont.	30 g/l	±% to cont.
Epidermis thickness	12.5	21.8	74.4	34.6	176.8
Cortex thickness	195.3	305.6	56.5	470.4	140.9
Fiber thickness	23.6	54.6	131.4	101.8	331.4
Phloem tissue thickness	17.3	38.9	124.9	41.6	140.5
Xylem tissue thickness	109.8	175.0	59.4	198.7	81.0
Vessel diameter	43.3	45.3	4.6	67.9	56.8
Pith diameter	1506.9	2036.8	35.2	2310.0	53.3
Stem diameter	2332.3	3167.5	35.8	3950.1	69.4



Details: ep, epidermis; co, cortex; ph, phloem; xy, xylem and pi, pith

Fig 1: Transverse sections of the median internode of the main stem of *Hibiscus sabdariffa* L plant. control (A), plant treated with henna aqueous extract 10g/l (B) and 30g/l (C).

The leaf anatomical parameters

It is observed from the data in Table 6 and Figure 2 that henna aqueous extract at a concentration of 30 g/l had the thickest blade (261.9 µm) when compared with the control and henna aqueous extract at a concentration of 10 g/l. This is because it contained the thickest upper and lower epidermis as well palisade and spongy tissues with increases of 37.8, 4.0, 41.2, and 91.2 %, respectively, compared to the control. In contrast, the lowest increase in blade thickness (169.2 µm) was recorded when plants were treated at a concentration of 10 g/l of henna aqueous extract with an increase of 2.5 % related to

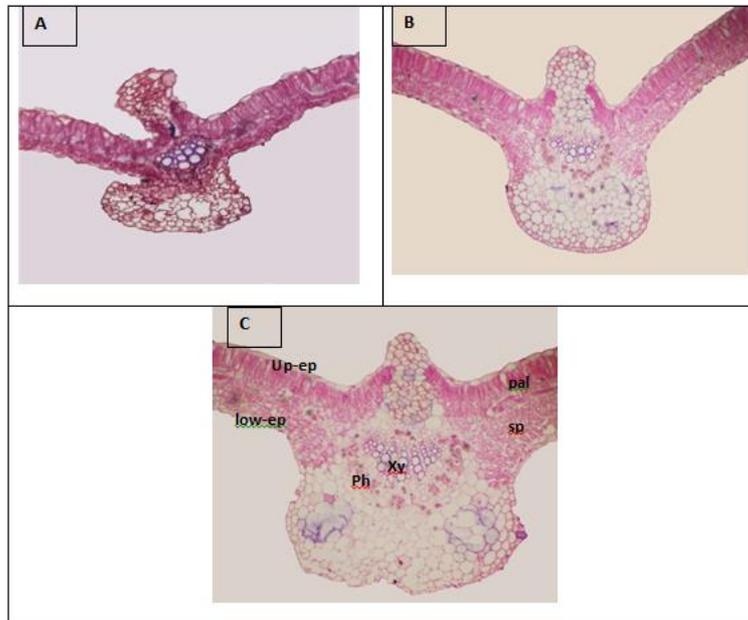
the control, due to the increase in thickness of both palisade (7.7 %) and spongy (8.6 %) tissues. Likewise, the 30 g/l concentration of henna aqueous extract also recorded the greatest thickness of midvein (989.3 µm). This is because it had the largest thickness of parenchyma above and below the main vascular bundle, xylem and phloem tissues, as well as No. of xylem vessels, compared to the other treatments, with increases of 85.5, 60.0, 67.2, and 121.1 % for the four mentioned tissues, respectively, compared to the control. However, the control scored the highest average diameter of vessel (30.6 µm).

Table 6: Effect of different henna extract concentrations on leaf anatomical characters of *Hibiscus sabdariffa* L plants.

Leaf anatomical characters (µm)	Treatments (henna extract)				
	control	10 g/l	±% to cont.	30 g/l	±% to cont.
Blade thickness	165.0	169.2	2.5	261.9	58.7
Upper epidermis thickness	18.0	15.6	-13.3	24.8	37.8
Lower epidermis thickness	15.1	12.4	-17.9	15.7	4.0
Palisade tissue thickness	60.0	64.6	7.7	84.7	41.2
Spongy tissue thickness	72.5	78.7	8.6	138.6	91.2
Midvein thickness	629.6	737.5	17.1	989.3	67.2
Xylem tissue thickness	81.7	65.4	-20.0	130.7	60.0
No. of xylem vessels	19.0	25.0	31.6	42.0	121.1
Vessel diameter	30.6	20.4	-33.3	25.5	-16.7
Phloem tissue thickness	23.5	19.6	-16.6	39.3	67.2
Thick. of parenchyma above and below the main vascular bundle	430.8	658.0	52.7	799.0	85.5

It is concluded from the foregoing results that, the stem anatomical measurements of the roselle plants shown in Table 5 exhibited a gradual increase in the stem anatomical parameters, starting from the low concentration of henna

aqueous extract (10 g/l) to a concentration of 30 g/l, as the increase was the highest, compared to the control. The anatomical structure of the leaf followed approximately the same previous direction in the stem Table 6.



Details: Up-ep, upper epidermis; pal, palisade tissue; sp, spongy tissue; low-ep, lower epidermis; xy, xylem; ph, phloem.

Fig 2: Transverse sections of the leaf blade developed at the median portion of the main stem *Hibiscus sabdriffa* L. plant. control (A), plant treated with henna aqueous extract 10g/l (B) and 30g/l (C).

Discussion

In this study, the effect of henna aqueous extract as natural stimulator at concentrations (0, 10, 20, 30 and 40 g/l) on roselle plants were discussed. Results indicated that foliar spraying with aqueous extract significantly increased growth, yield components and quality of roselle plants compared to control. This may be due to the fact that henna aqueous extract contain deferent concentrations of bioactive compounds as shown in Table 1. Bio-stimulants have the ability to enhance flowering, fruiting, yield and production of an important secondary metabolites [14]. IAA has an important role in enhancing apical bud dominance, root initiation, delay in abscission, vascular differentiation and fruits development [15]. In this respect, the positive effect of GA₃ (gibberellic acid) on growth, seeds and fruits production was recorded by [16]. Furthermore, [17] reported that GA₃ increased the biosynthesis of anthocyanin.

In this regard, phenolic compounds are an important class of secondary metabolites, which are involved in many physiological processes such as seed germination, cell division and synthesis of photosynthesis pigments [18]. Similarly, they improve nutrient uptake through chelation of metallic ions and stimulated absorption [19]. These compounds act as natural phytohormones, antioxidants as well as they have antimicrobial activity [20]. On the other hand, some phenolic compounds may be potentially phytotoxic if accumulated in high concentration which can inhibit seed germination and growth [21] such effect attributed to the disruption of cellular enzyme functioning and impairment cell division [22].

The positive effect of lawsone (2-hydroxyl-1-4 naphthoquinone) on roselle plants growth may be due it has antibacterial, antifungal activity and antioxidant effect [23-24]. Also, flavonoids are vital secondary compounds, as they help in pollination process [25]. Moreover, these flavonoids are conjugated to other flavonoids and glucose to produce anthocyanin and flavonol glycoside [26]. As for alkaloids, they were considered waste of products of nitrogen store and were revealed as plant growth regulators [27]. Also, alkaloids have physiological properties and toxicological. Stimulation or inhibition depends on dose, an increase in concentration

above optimal level lead to phytotoxic [28].

In the anatomical study, noteworthy to mention that, most treatments of henna aqueous extract improved anatomical characteristics of the stem and leaf of the roselle plant. This may be due to henna extract contains, flavonoids, carbohydrates, alkaloids, tannins, protein, quinines, and coumarins. The most effective concentration is 30 g/l. It recorded the highest values for most parameters of stem and leaf. The major active constituent in henna is 2-hydroxy-1-4 naphthoquinone [4]. This component may affect tissue, such as flavonoids play a variety of biological activities in plants. Flavonoids have long been known to be synthesized in particular sites and play a vital role in the growth and development of seedling [29] as henna aqueous extract contains different concentrations of bio- growth regulators such as IAA and GA₃.

IAA increased cell division and accumulation of building units accompanied by greater polysaccharides and total carbohydrates contents [30], IAA showed an increase in the diameter, area and the number of leaves was also observed by [31].

Gibberellins stimulated stem growth by promoting both cell elongation and cell division [14]. [16] reported that GA₃ growth regulators are effective in developmental strategies as seed germination, stem elongation, leaf growth, [32] on black iris. They all concluded that gibberellic acid is utilized to regulate plant increase through the increasing cellular portion and cell elongation [33]. Foliar treatment of IAA and/or GA₃ caused marked increases in growth parameters of mungbean by [34].

It could be concluded that spraying roselle plants with henna aqueous extract at 30 g/l was superior to high concentration (40g/l) in increasing growth and productivity. This result may be due to henna extract contains secondary metabolites compounds including phenolic, flavonoids and alkaloids compounds, which they have the ability as stimulator or inhibitor of plant growth.

Recommendation

Foliar application of henna aqueous extract at 30 g/l could be recommended to obtain highest growth, yield and calyces quality of roselle plant.

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