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Antioxidant Properties, Secondary Metabolites and Yield as Affected by Application of Antioxidants and Banana Peel Extract on Roselle Plants

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ABSTRACT

Two field experiments were carried out during two successive seasons (2013 and 2014) at the Experimental Station of National Research Centre, Nubaria district, Beheira Governorate, Egypt to compare the physiological role of foliar application of different antioxidants as tryptophan at 50 &100 mg/l, potassium (potassium nitrate) at 100 mg/l, t-cinnamic acid (200 & 400 mg/l) and banana peel extract at 5% and 10% concentrations on yield quantity and quality of Roselle sepals. All treatments significantly increased yield components such as number of fruits/plant, fresh & dry weights of fruits/plant, fresh and dry weights of sepals/plant as compared with those of the untreated plants. Banana peel extract was the most effective treatment followed by t-cinnamic acid followed by tryptophan then potassium nitrate. Different treatments increased significantly activities of phenylalanine ammonialyase (PAL) and tyrosine ammonialyase (TAL) enzymes, phenolic and anthocyanins contents of Roselle leaves. Various treatments increased significantly phenolics and nutritive phytochemical components (ascorbic acid, anthocyanins, flavonoids, tannins) and antioxidant activities of sepals, while they mostly decreased titratable acidity as compared with control plants. Different treatments increased markedly total soluble sugars and different sugar fractions as compared with those of the control plants. It is worthy to mention that banana peel extract treatments had more pronounced effect than other different treatments.

KEY WORDS
Anthocyanins, Antioxidants, Banana peel extract, Flavonoids, Roselle, Sepals, Tryptophan.

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) Family Malvaceae is a medicinal plant, commonly known as Roselle, red sorrol, or karkadeh is widely grown in Africa, South East Asia, and some tropical countries of America. Roselle is cultivated in Egypt throughout the country from North to South, although the Southern regions are more suitable for its cultivation. However, the new reclaimed soils are suitable for such plants, which are able to grow under different climatic conditions. Roselle is an annual herbaceous shrub that has many industrial, pharmaceutical uses in many countries all over the world and utilized beyond these areas of cultivation globally [28]. *Hibiscus sabdariffa* L. is cultivated for its stems, leaves, calyces and seeds as all parts have industrial, medicinal and other applications. Sepals are the most important economic parts of Roselle plants [32]. Fresh juicy and even dried calyces used in preparation of beverages, jams, jellies, sauces, cakes, puddings, syrup and wine [6]. Karkadeh considered a very popular beverage and valuable medicinal plant due to its effect on lowering and/or adjusting blood pressure (anti-hypertension) without production of any side effects [27]. In addition, it has an effect on stomach function, and can resist various infections of intestinal disease [60]. [53] added that, *Hibiscus sabdariffa* flowers could be used to relax the pain of muscles of uterus and intestine. It has highly antibacterial properties. As well as, is considered cardiotonic. It is useful as laxatives [33].Also, [78] stated that protocatechuic acid (a simple phenolic compound) detected in *Hibiscus sabdariffa* could be used to fight pyrexia and liver disorders. Also, it has been demonstrated that this compound is an effective agent in reducing the carcinogenic action of diethylnitrosamine in the liver. Phytochemical studies showed the presence of alkaloid, flavonoids, ascorbic acid, tannin, polyphenols, anthocyanin, oxalate and saponin [36].

Egypt suffers from food shortage problem as a result of a huge increment of population and the huge loss of agricultural soils due to desertification and erosion problems. Therefore, it is very essential to increase plant productivity. So great attention has paid to the newly reclaimed soil outside the Nile Valley, which represent about 3-4% of the total area of Egypt. The newly reclaimed soil is mostly exposed to a combination of environmental stress conditions including low water availability, nutrient deprivation, temperature fluctuations and high irradiances. In this concern, great efforts must be paid to increase plant tolerance to such conditions via selecting tolerant genotypes and applying the optimum cultural practices and/or treating the seeds (before sowing) or plants (at different growth stages) with some growth regulating substances or antioxidant compounds that play an important role for helping plants to overcome partially the unfavorable conditions and avoid their negative effects on crop yield quantity and quality. So, Roselle production can be increased by two means, either by horizontal expansion (cultivation in newly reclaimed lands) or by vertical expansion (using antioxidant or growth regulators).

Plant wastes are freely available on most organic holdings and these can be composted for the supply of nutritious organic matter to be returned to the soil or extracted by simple methods to obtain natural antioxidants. Significant quantities of banana or plantain peels, equivalent to 40% of the total weight of fresh banana, are generated as a waste product in industries producing banana based products [80]. At present, these peels are not being used for any other purposes and are mostly dumped as solid waste at large expense. It is thus significant and even essential to find applications for these peels as they can contribute to real environmental problems. Potential applications for banana peel depend on its chemical composition. Banana peel is rich in dietary fibre, proteins, essential amino acids, polyunsaturated fatty acids and potassium [25]. Banana peel also demonstrated the presence of various phenolic compounds, antioxidants as vitamins and K element. These facts seem to suggest that extracts from many different kinds of plant materials as banana peel contain common growth promoting substances, which may be involved (as foliar or soil applications) in the mechanism of induction of growth in various kinds of plant species [7, 37].

Antioxidants are considered as bio-regulator compounds, which relatively in low concentrations influence plant growth by regulating many physiological processes, such as synthesis of enzymes, act as co-enzymes, added to protect plant from harmful effects of stresses [90]. Antioxidants could be used as a potential growth regulator to improve plant growth and yield and increase plant resistance to such environmental stress conditions of newly reclaimed sandy stress. Antioxidants as amino acids and phenolic acids are designing chemicals, when added in small quantities maintain normal growth and proper development of all plants. Amino acids as organic nitrogenous compounds are the building blocks in the synthesis of proteins [17]. Amino acids are particularly important for stimulation cell growth, they act as buffers which help to maintain favorable pH value within the plant cell, since they contain both acid and basic groups; they remove ammonia from the cell. This function is associated with amid formation, so they protect plants from ammonia toxicity. They can serve as a source of carbon and energy, as well as protect plants against pathogens. L-tryptophan known to be a physiological precursor of auxins in higher plants. It is investigated that L-tryptophan has more positive effect on plant growth and yield as compared to pure auxins [91]. L-tryptophan is an amazing amino acid. It may act as an osmolyte, ion transport regulator, modulates stomatal opening and detoxify harmful effects of heavy metals [56, 64].

Potassium (K) is another important nutrient that has favorable effects on most of the biochemical and physiological processes necessary for plant growth such as cell wall development, metabolism of nucleic acids, proteins, vitamins and growth substances [71]. Furthermore, Potassium (K^+) ions play vital roles in carbon assimilation, photosynthesis, translocation of photosynthates and the synthesis -and translocation of organic and inorganic nutrients from the soil to the plant [82]. Also, potassium plays a vital role in: control of ionic balance, regulation of plant stomata and water use, activation of plant enzymes and, many other processes [46, 66]. Potassium is not only an essential macronutrient for plant growth and development, but also is a primary osmoticum in maintaining low water potential of plant tissues.

Phenolics are low molecular compounds ubiquitous in all tissues of higher plants with great significance in plant development. Phenolic compounds are some of the most widespread molecules among plant secondary metabolites, and are of great significance in plant development [15]. Furthermore, these biomolecules may contribute to soil and water conservation, weed management, mineral element nutrition, as well as they impact as signal molecule in certain symbiotic relationships, and act as defense molecules against soil pests and pathogens [45]. Additionally, they serve as flower pigments; act as constitutive protection agents against biotic and abiotic stress [20]. Trans-cinnamic acid is one of phenolic acids, the role of trans-cinnamic acid as phenolics in stimulating growth and activating plants was studied by many investigators who reported that plants synthesize large amounts of phenylpropanoid acids, mainly hydroxycinnamic acids and hydroxybenzoic acid [21]. In addition, cinnamic acid is a key intermediate in shikimate and phenylpropanoid pathways. Shikimic acid is a precursor of many alkaloids, aromatic amino acids, and indole derivatives. It is found both in free form, and especially in the form of esters (ethyl, cinnamyl, benzyl), in various essential oils, resins and balsams etc. These are very important intermediates in the biosynthetic pathway of most of the aromatic natural products. These are widely spread in the plants and possess wide range of activities. Trans-cinnamic acid has a long history of human use as a component of plant-derived scents and flavourings [34]. It belongs to the class of auxin, which is recognized as plant hormones regulating cell growth and differentiation [83]. Cinnamic acid functionality is also present in a variety of secondary metabolites of phenylpropanoid biosynthetic origin [26].Therefore, the present study was undertaken to compare between the physiological roles of some antioxidants as L-tryptophan, potassium and trans- cinnamic acid (as a chemical antioxidants) and banana peel extract (as a natural source of antioxidants) on some biochemical aspects, yield and some secondary metabolites and antioxidants of sepals of Roselle plant grown under newly reclaimed sandy soils.

MATERIALS AND METHODS

Plant material and growth conditions:

A field experiment was conducted at the Experimental Station of National Research Centre, Nubaria district Beheira Governorate, Egypt, during summer season of 2013 and 2014. The physical and chemical properties of the experimental soil sites were analyzed (Table 1) according to [13].

Table 1: Physical and chemical analysis of the experimental soil sites.

A. Physical analysis:

Sand		Silt 20-0µ%	Clay < 2µ%	Soil texture
Course 2000-200µ%	Fine 200-20µ %			
47.46	36.19	12.86	4.28	Sandy

B. Chemical analysis:

pH 1:2.5	EC dSm^{-1}	$CaCO_3$	OM%	Soluble cations meq/l				Soluble anions meq/l			
				Na^+	K^+	Mg^+	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
7.60	0.13	5.3	0.06	0.57	0.13	0.92	1.0	0.0	1.25	0.48	0.89

Available nutrients						
Macro elements (ppm)		Micro elements (ppm)				
N	P	K	Zn	Fe	Mn	Cu
52	12.0	75	0.14	1.4	0.3	0.00

Seeds of Roselle *Hibiscus sabdariffa* L. (dark colored) cultivar were obtained from Agricultural Research Centre Giza, Egypt. The experimental design was in randomized complete block with four replications, Roselle (*Hibiscus sabdariffa* L.) seeds were sown on the 7th May in both seasons at the rate of 60 kg/faddan (one faddan =0.42 ha) in rows 3.5 meters long, and the distance between rows was 20 cm apart. Plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length). The recommended agricultural practices of growing Roselle were applied. Pre-sowing, 150 kg/feddan of calcium super-phosphate (15.5% P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at a rate of 75 Kg/feddan in five equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulfate (48.52 % K_2O) was added in two equal doses of 50 kg/feddan, before the 1st and 3rdirrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. In both seasons, foliar application of different treatments was applied as follow:

- Control (treated with water)
- Tryptophan (50 mg/l)
- Tryptophan (100 mg/l)
- Potassium nitrate (100 mg/l)
- Trans-cinnamic acid (100 mg/l)
- Trans-cinnamic acid (200 mg/l)
- Banana peel extracts (5%)
- Banana peel extracts (10%)

Different treatments were carried out twice; where plants were sprayed after 45 and 60 days from sowing. Plant samples were taken after 75 days from sowing for estimation of some biochemical parameters in leaves of plant such as indole acetic acid (IAA), phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) enzymes, phenolics and anthocyanins contents. At harvest, the following characters were recorded at random samples of 10 girded plants in each plot to estimate the following characters: number of fruits/plant, fresh & dry weights of fruits/plant, fresh and dry weights of sepals/plant. Some biochemical contents of the yielded sepals such as phenolics, ascorbic acid, anthocyanins, flavonoids, tannins, antioxidant activities, titratable acidity, citric acid & oxalic acid contents and total soluble sugars and its fractions were determined.

Extract preparation:

Banana peels were air dried at room temperature for three weeks to obtain consistent weight. The dried peels were later ground to powder. The ground peels materials were shaken separately in methanol for 48 hrs on a shaker at room temperature. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. The extract was resuspended in water to yield a 5% and 10% concentrations [79].

Biochemical analysis:

Indole acetic acid content were extracted and analyzed by the method of [41]. The extraction and assay of phenylalanine ammonia lyase (PAL, EC, 4.3.1.1) and tyrosine ammonia lyase (TAL, EC, 4.3.1) enzymes were carried out according to the method adopted by [10]. Phenolic contents were extracted and then measured as described by [16]. Anthocyanins of leaves or sepals were estimated according to [84]. Ascorbic acid was determined by the method of [65]. Total flavonoids were determined using the method reported by [12]. Tannins were determined using the modified vanillin hydrochloric acid (MV-HCl) as reported by [47]. The free radical scavenging activity was determined according to [43] using the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent. The titratable acidity of sepals was determined as citric acid by the method of titration against alkali [8]. The extracted soluble sugars were analyzed using HPLC according to the method of [54], Aminex Hp x -87H column, 300 x 7.8 mm at 0.6 ml/min flow rate at 85 ^{o}C, detection: RI 16 x standard of galactouronic acid. The galactose (Gal.), glucose (Glu.), rhamnose (Rham.) and mannitol (Mannit.) were used.

Statistical analysis:

The data obtained were statistically analyzed according to [73]. The combined analysis of the two growing seasons was carried out according to [74]. Means were compared by using Least Significant Difference (LSD) at 5% levels of probability.

Results:

Yield and yield components:

Data presented in Table 2 show the effect of foliar application of tryptophan (50 and 100 mg/l), potassium (K) (100 mg/l), or t-cinnamic acid (100 and 200 mg/l) and banana peel extract (5% and 10%) on yield parameters of Roselle plants grown under newly reclaimed sandy soil. Data clearly show that, application of different antioxidants increased significantly yield and yield components such as number of fruits/pant, fresh and dry weights of fruits and sepals/plant (g) as compared to untreated plants. The maximum increases of the yield parameters were obtained by foliar application with banana peel extract followed by t-cinnamic acid. Banana peel extract with 5% concentration was more effective than 10% as compared with control plants. As the percentage of increases in response to 5% banana peel extract reached to 99.93%, 90.73%, 106.72, 81.42 and 87.63% in comparison to 92.61%, 73.30%, 72.25%, 63.72 and 77.76% in response to 10% banana peel extract treatment for number of fruits/plant, fresh & dry weights of fruits/plant and fresh & dry weights of sepals/plant as compared to the untreated plants, respectively.

Table 2: Effect of tryptophan, K, t-cinnamic or banana peel extract, on yield parameters of Roselle plant grown under newly reclaimed sandy soil (Data are means of two seasons).

Treatment		Number of fruits/plant	Fresh weight of fruits/plant (g)	Dry weight of fruits/plant (g)	Fresh weight of sepals/plant (g)	Dry weight of sepals/plant (g)
Control		13.67	80.35	19.06	28.25	8.41
Tryptophan	50 mg/l	18.67	106.35	23.26	37.52	10.77
	100 mg/l	21.67	114.52	28.04	37.58	12.67
K 100 mg/l		18.33	111.25	20.73	38.32	10.78
t-cinnamic acid	100 mg/l	22.33	115.32	27.53	38.25	10.14
	200 mg/l	24.67	126.25	30.63	43.52	11.00
Banana peel extract	5%	27.33	153.25	39.40	51.25	15.78
	10%	26.33	139.25	32.83	46.25	14.95
LSD (0.05)		3.61	11.35	3.63	3.57	1.50

IAA contents:

The changes in indole acetic acid (IAA) contents of Roselle leaves in response to different treatments are presented in Table 3. Data clearly show the promotive effect of foliar application with different antioxidants concentrations used (tryptophan, K and t-cinnamic acid) in addition to banana peel extract as compared with control plants. Also, foliar spraying with banana peel extracts was the most effective treatment followed by t-cinnamic acid treatment. Low concentration of banana peel extract (5%) was more effective than high concentration (10%), meanwhile high concentrations of tryptophan or t-cinnamic acid (100 & 200 mg/l) were more effective than low concentrations (50 & 100 mg/l), respectively.

Table 3: Effect of tryptophan, K, t-cinnamic or banana peel extract on IAA contents, PAL and TAL enzyme activities, phenolic and anthocyanins contents of Roselle leaves grown under newly reclaimed sandy soil.

Treatment		IAA (µg/100g fw)	PAL (µg t-cinnamic acid/g fw/min.)	TAL (µg p-coumaric acid/g fw/min.)	Phenolics (mg/g fw)	Anthocyanins (mg/g fw)
Control		15.50	6.99	13.52	18.09	4.06
Tryptophan	50 mg/l	17.60	11.47	25.53	20.34	4.65
	100 mg/l	21.98	13.89	32.43	21.73	4.98
K 100 mg/l		20.53	11.03	28.08	21.62	4.65
t-cinnamic acid	100 mg/l	19.82	14.03	30.78	22.37	5.32
	200 mg/l	25.05	16.47	37.88	22.69	6.06
Banana peel extract	5%	32.28	20.96	40.38	23.54	6.65
	10%	26.60	17.55	38.54	23.12	6.48
LSD (0.05)		1.93	0.63	1.29	0.76	0.52

Activities of PAL and TAL enzymes:

Phenyl alanine ammonialyase (PAL) and tyrosine ammonialyase (TAL) activities were increased in response to application of different concentrations of tryptophan, K, t-cinnanic acid or banana peel extract on Roselle plants as compared to those of untreated control plants. The most effective treatment was detected with banana peel extracts followed by treatment with t-cinnamic acid. Regarding to banana peel extract 5% treatment was more effective concentration than 10% as it increased PAL and TAL activities by 199.86% and 198.67%, respectively, meanwhile the increases reached to 151.07% and 185.06% in response to 10% banana peel extract as compared to control plants.

Phenolic and anthocyanins contents:

Data presented in Table 3 revealed that, treatment of Roselle plant with different concentrations of tryptophan (50 &100mg/l), K (100 mg/l), t-cinnamic acid (100 & 200 mg/l) or banana peel extract (5% & 10%) significantly increased phenolic and anthocyanins content of Roselle leaves as compared with control plants. The most effective treatment was banana peel extract with 5% as compared with control plant.

Phenolic and nutritive phytochemical components of the yielded sepals:

Special attention must be paid to the phenolic contents and nutritive phytochemicals (ascorbic acid, anthocyanins, flavonoids and tannins) of the yielded Roselle sepals to determine its nutritive value. Data presented in Table 4 demonstrated that tryptophan (50 &100 mg/l), K (100 mg/l), t-cinnamic acid (100 & 200 mg/l) or banana peel extract (5% & 10%) treatments significantly increased phenolic, ascorbic acid, anthocyanins, flavonoids and tannins contents. The highest significant increase was obtained with banana peel extract followed by t-cinnamic acid. Also, data showed that, higher concentrations of tryptophan (100 mg/l) and t-cinnamic acid (200 mg/l) were more effective than lower concentrations 50 mg/l for tryptophan and 100 mg/l for t-cinnamic acid. In the meantime, lower concentration of banana peel extract 5% was more effective than higher concentration 10%, except for tannins 10% concentration increased tannins contents more than 5% concentration.

Table 4: Effect of tryptophan, K, t-cinnamic or banana peel extract, on phenolic and nutritive phytochemical components of the yielded sepals of Roselle plant grown under newly reclaimed sandy soil.

Treatment		phenolics (mg/g dw)	Ascorbic acid (mg/g fw)	Anthocyanins (mg/g dw)	Flavonoids (mg/g dw)	Tannins (mg/g dw)
Control		28.86	18.35	15.15	13.12	0.44
Tryptophan (mg/l)	50	30.06	25.35	18.35	17.15	0.49
	100	32.91	36.35	21.45	20.61	0.68
K 100 (mg/l)		31.12	29.25	19.47	21.67	0.53
t-cinnamic acid (mg/l)	100	34.50	29.25	21.57	21.46	0.65
	200	35.49	33.35	28.67	36.34	0.75
Banana peel extract	5%	45.50	35.25	31.96	32.48	0.68
	10%	41.06	38.35	29.74	30.86	0.75
LSD (0.05)		0.15	2.58	1.16	2.01	0.012

Antioxidant activity:

The radicals scavenging activity of Roselle sepals extract, as shown in Table 5 expressed the percentage reduction of the initial DPPH absorption by the tested antioxidants. Different treatments with different concentrations increased significantly antioxidant activities at 50 and 100µg/ml. Banana peel extract (5% &

10%) were the most effective treatment followed by t-cinnamic acid followed by tryptophan then K. Banana peel extracts with lower concentration (5%) was more effective than higher concentration (10%). Meanwhile higher concentrations of t-cinnamic acid (200mg/l) and tryptophan (100 mg/l) were more effective than lower concentrations (100 and 50 mg/l) for t-cinnamic acid and tryptophan respectively.

Table 5: Effect of tryptophan, K, t-cinnamic or banana peel extract on antioxidant activity at different concentrations (50 and 100 µg/ml) of the yielded sepals of Roselle plant grown under newly reclaimed sandy soil.

Treatment		Antioxidant %	
		50 µg/ml	100µg/ml
Control		32.65	53.58
Tryptophan (mg/l)	50	38.27	70.25
	100	43.81	79.35
K (100 mg/l)		36.81	68.35
t-cinnamic acid (mg/l)	100	43.37	72.25
	200	46.18	78.58
Banana peel extract	5%	49.76	82.35
	10%	47.61	79.65
LSD (0.0 5)		1.94	3.25

Titratable acidity and acids contents:

Data presented in Table 6 show the effect of foliar treatment of tryptophan (50 & 100 mg/l), K (100 mg/l), t-cinnamic acid (100 & 200 mg/l) and banana peel extract (5 & 10%) on titratable acidity and acid contents as citric and oxalic acids. Different treatments caused variable changes in titratable acidity, citric acid and oxalic acid of sepals of Roselle plants. Tryptophan treatment caused gradual increases in titratable acidity, while banana peel extract at 5% increased significantly citric acid content. In the meantime, all studied treatments almost decreased significantly titratable acidity and acid contents as oxalic and citric acids.

Table 6: Effect of tryptophan, K, t-cinnamic or banana peel extract, on titratable acidity, citric acid and oxalic acid (mg citric/100g dw) of Roselle plant grown under newly reclaimed sandy soil.

Treatment		Titratble acidity	Citric acid	Oxalic acid
Control		17.34	8.84	5.90
Tryptophan (mg/l)	50	18.54	7.90	5.49
	100	19.26	7.45	4.16
K (100 mg/l)		16.37	7.22	4.65
t-cinnamic acid mg/l	100	16.07	6.73	4.01
	200	15.51	8.14	5.75
Banana peel extract	5%	16.81	9.41	5.81
	10%	15.51	8.59	5.49
LSD (0.05)		0.83	0.41	0.42

Total soluble carbohydrates of sepals:

Table 7 show the effect of different concentrations of tryptophan, potassium, t-cinnamic and banana peel extract on total soluble sugars of Roselle sepals. Different treatments increased total soluble sugars as well as different sugars as galactouronic (Gal), gluocose (Glu), rhaminnose (Rham) and mannitol (Mannit). The most effective treatment was banana peel extract followed by t-cinnamic acid. Low concentration (5%) of banana peel extract was more effective than high concentration (10%). Meanwhile high concentrations of t-cinnamic acid (200 mg/l) or tryptophan (100 mg/l) were more effective than lower concentrations (100 mg/l & 50 mg/l), respectively.

Table 7: Effect of tryptophan, K, t-cinnamic or banana peel extract, on soluble sugars (g/100g dw) of water extract of sepals of Roselle plants grown under newly reclaimed sandy soil.

Treatment		Gal	Glu	Rham	Mannit	TSS
Control		34.75	6.74	1.45	0.41	43.35
Tryptophan (mg/l)	50	37.25	6.02	1.42	0.75	45.44
	100	42.52	7.02	1.54	0.79	51.87
K (100 mg/l)		40.25	7.42	1.15	0.54	49.36
t-cinnamic acid (mg/l)	100	47.25	6.85	1.02	0.65	55.77
	200	49.75	7.52	1.45	0.74	59.46
Banana peel extract	5%	54.35	7.65	2.01	0.75	64.76
	10%	50.35	6.68	1.75	0.52	59.31

Discussion:

Banana peel represents about 40% of total weight of the fresh fruit. The total amount of phenolic compounds in banana peel has been reported from 0.90 to 3.0 g/100g dry weight. Chemical analysis of banana peel extract shows that, banana peel extract has high contents of vitamin A as beta carotene, vitamin C, amino acids especially tryptophan, protein, carbohydrates, macro and micronutrients, phenolic compounds, fat and fibers [52]. Thus, it is clear that all banana peel extract components have simulative effect are very important for the influence on plant growth and yield by regulating many physiological processes. Exogenous application of tryptophan, K, t-cinnamic acid and banana peel extract significantly increased plant productivity (Table 2), IAA contents, PAL and TAL enzyme activities, phenolic and anthocyanins contents of Roselle leaves (Table 3). In addition, different treatments increased phenolic and nutritive phytochemical components of Roselle sepals (Tables 4) Antioxidant activities of sepals at different concentrations (50 and 100 µg/ml) also were increased in response to different treatments (Table 5). Meanwhile, different treatments caused variable changes in titratable acidity & citric and oxalic acid contents of sepals in Roselle plants (Table 6). Total soluble sugars and sugars fractions of sepals of Roselle plants were increased in response to different treatments (Table 7). With regard to the increases in yield components of Roselle plant in response to tryptophan treatment, these results were confirmed by the findings of [18, 23, 31, 56], they stated that tryptophan treatment increased yield components and some biochemical constituents of canola, snap bean, wheat and thyme plants, respectively. Amino acids such as tryptophan which have a high integrity with different metabolic pools in plants were used to promote plant growth and yield of plants [14]. [87] reported that the regulatory effects of tryptophan could indirectly be explained since tryptophan was suggested to affect plant development through their influence on auxin biosynthesis (Table 3). Regarding to potassium, generally the essential element K has a great regulatory role within plant cells and organs such as, activating more than 50 enzymes, osmosis regulation and photosynthesis and loading and unloading of sugars in phloem [48]. [38, 39, 40, 81] obtained similar results for fodder beet, tomato, strawberries and mungbean plants, respectively. The element K has important regulatory roles inside and outside of plant cells, so it can be expected that plant access to an available K source should optimize plant growth and consequently this enables plants to flower more frequently. Also, the promotive effect of K treatment on yield components may be due to the fact that potassium plays an important role in promoting synthesis of photosynthates and their transport [48]. On harmony with our obtained results of t-cinnamic acid [1, 19, 58, 59] using phenolic acid as salicylic acid on flax, sunflower, tomato and faba bean plants. In addition, [76] stated that, t-cinnamic acid treatment increased yield components of Khella plant. Concerning the stimulatory effect of phenolic acids as t-cinnamic acid on sepals yield components (Table 2) it may be attributed to the effect of these compounds on many biochemical and physiological processes that were reflected on improving vegetative growth and active translocation of the photosynthesis products from source to sink. With regard to banana peel extract effect, many efforts have addressed growth-regulating compounds to be good for inducing growth with low cost natural extracts. Optimum plant growth and development is important for greater final dry matter and yields. In order to achieve this, sufficient amounts of nutrients should be applied through organic and inorganic sources. The extracts from many different kinds of plant materials as banana peel promote the yield of Roselle plant that may be these extracts contain common growth promoting substances involved in the mechanism of induction of growth and yield of plants [63]. Also, the promotive effect of banana peel extract may be due to the presence of antioxidans, phenolic compounds, macro and micronutrients. Also, low concentration 5% was more effective than high concentration 10% as it caused the highest increases in yield components this may be due to that, the low concentration has the most suitable concentrations of antioxidant and phenolic contents.

Regarding to IAA contents of Roselle leaves, [77] demonstrated that tryptophan applied to periwinkle plants increased IAA concentration. Also, [31] confirmed the obtained result on wheat plant. These increases of IAA content may be attributed to the conversion of tryptophan to IAA as stated by [62]. Regarding to t-cinnamic acid, It is well known that phenolic compounds as t-cinnamic acids induces flowering, increases flower life, retard senescence and increases cell metabolic rate. In addition, it may be a prerequisite for the synthesis of auxin [29, 49, 58]. Furthermore, these increments in growth regulating substances (IAA) in response to different treatments might be a prerequisite for acceleration of growth resumption of Roselle plant. These increases could be attributed to the increases in their biosynthesis and/or decrease in their degradation and conjugation. The increases in IAA in leaves tissues of Roselle plant concurrently with increasing growth rate and sepals yield due to the role of these endogenous hormones in stimulating cell division and/or the cell enlargement and subsequently growth [75 .[

With regard to PAL and TAL enzymes, anthocyanins and phenolic contents of Roselle leaves in the present study, it could be deduced that there has been a close relationship between the higher activities of PAL and TAL and the higher levels of anthocyanins and phenol contents of Roselle plants (Table 3). PAL is one of the key enzymes in controlling anthocyanins biosynthesis from phenylalanine. Also, TAL, another enzyme involved in phenols synthesis [85]. PAL enzyme catalyzes the elimination of ammonia from phenylalanine to give trans-cinnamic acid. This reaction is the first step of phenyl propanoid pathway in plants which results in the diversion of L-phenylalanine into secondary metabolism with subsequent production of anthocyanins, flavonoids and other phenolic compounds [32]. Many investigators confirmed our obtained results of phenol contents as reported by [42, 35, 51] using K foliar treatment on different plant species. Also, [19] on sunflower and [9] on flax using salicylic acid as phenolic acid. The increased accumulation of phenolic compounds was observed after different treatments may depend on the increases of PAL and TAL activities. PAL activity is known to be correlated with synthesis various phenolic compounds [55, 57, 70]. Also, these increases might be attributed to the increase in carbohydrate synthesis [70]. [18] showed that the increase in total phenolic contents was concur with increasing IAA contents and led to the suggestion that most of phenolic compounds are diphenols and polyphenols which may inhibit IAA oxidase activity and leading to auxin accumulation and reflected in stimulating the growth and yield of plant.

With respect to the phenolic and nutritive phytochemicals in methanolic extracts of *Hibiscus sabdariffa* sepals (Table 4). Phenols are one of the major groups of non-nutritive dietary. Different treatments increased significantly phenols and phytochemicals in the yielded sepals of Roselle plants. [30] stated that salicylic acid treatment increased total phenols of ginger plant [69] showed that benzoic & salicylic acid increased phenolic and flavonoids contents of soybean seeds. This stimulatory effect of different treatments might be attributed to their effects on enzymatic activity and translocation of the metabolites to Roselle sepals. The increases in anthocyanin contents in Roselle sepals resulted from the different treatments may be attributed to the increase in PAL and TAL activities in Roselle leaves (Table 3) concomitantly with increasing sucrose contents [70]. [22] confirmed this result in leaf disks of Indian almond treated with sucrose through inducing anthocyanins synthesis and phenylalanine ammonia lyase activity. [89] reported that the key enzyme PAL and chalcone synthase within the phenyl propanoid/flavonoid pathway. [85] suggested that phenylalanine was catalyzed to form cinnamic acid and ammonia through PAL enzyme. Cinnamic acid is known to be the parent compound for anthocyanin and lignin biosynthesis. Moreover, [24] found that Roselle plants accumulate anthocyanins by applying phenylalanine. The high polyphenol content in *Hibiscus sabdariffa* is of health benefit to consumers as a potential source of natural antioxidant. Anthocyanins are known to inhibit low density lipoprotein (LDL) oxidation and LDL-mediated macrophage apoptosis, serving as a chemo-preventive agent [36]. High contents of flavonoids has a very important effect on human health as flavonoids in human diet may reduce the risk of various cancers as well as prevent menopausal symptoms [61]. The importance of the flavonoids was known to possess significant antimicrobial activities and was utilized as natural plant protectants [88]. Regarding to tannins content, edible plant materials containing tannins are known to be astringent, and are used for treating intestinal disorders such as diarrhea and dysentery [72]. The presence of tannins in *Hibiscus sabdariffa* supports their use in traditional curing of many different diseases [36]. *Hibiscus sabdariffa* had ascorbic acid content ranged from 18.35 to 38.35 mg/g are very high in ascorbic acid content, and could serve as good food supplement for ascorbic acid, a nutritive phytochemical [36].

The 1, 1 diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with maximum absorbance at 517 nm and can readily undergo reduction by an antioxidant. Due to the ease and convenience of this reaction, it now has widespread use in the free radical-scavenging activity assessment [11]. The reduction of DPPH radical (purple colour) to a yellow coloured compound, diphenyl picrylhydrazine in Roselle sepal's extracts depends on hydrogen donating ability of the studied antioxidants and a dose dependent scavenging of DPPH free radical by sepals extracts from 50 to 100 µg/ml was observed. Tables 5 showed that the best radical scavenging activity percentages was obtained at 100 µg ml^{-1} with plants treated with 5% banana peel extract. Also, different treatments increased antioxidant activity as compared to untreated controls. These results using different antioxidants were also supported by [2, 67] on different orange cvs, they realized that the antioxidant scavenging potential was directly proportional with the concentration of the used samples (12.5 to 100µl). Moreover, [58] using salicylic acid as a phenolic compound caused significant increases in the antioxidant activity in tomato fruit extracts at the two used samples (40 and 80µl) relative to control plants. These results may be due to the effect of different treatments (t-cinnamic acid and banana peel extract as phenolic compound derivatives) as precursor for the synthesis of anthocyanidin (flavonoid structure) and they correlated with the increasing anthocyanin and its antioxidant activity [5].

With respect to titratable acidity, citric and oxalic acids contents of the yielded Roselle sepals, different treatments caused variable changes of titratable acidity, citric and oxalic acids (Table 6). The increase in the acid contents of sepals may be attributed to the increase in catabolic activities, while the decrease in the acid contents of sepals of the differently treated Roselle plant may be attributed to be due to the shift of sugars and certain catabolic intermediates to the developing sepals where they incorporated into the biosynthesis of carbohydrates [70]. Moreover, the decline in the acid contents in the sepals of the differently treated Roselle plants may be due to the increased rate of the catabolism more than anabolism. It has been also shown that the different treatments used (Tryptophan, K, t-cinnamic and banana peel extract) had significant effects on total soluble sugar levels of Roselle sepals at harvesting as compared with those of the control (Table 7). Similar results were obtained on different plants reported by [4, 56] using tryptophan on different plant species. The promotive affect of the amino acid tryptophan on TSS contents may be due to their important role on the biosynthesis of chlorophyll molecules which in turn affected chlorophyll content [3]. Regarding to phenolic acids, [9, 30] stated that salicylic acid treatment increased TSS of flax and ginger plants, respectively. Also, [76] showed that trans-cinnamic acid foliar treatment increased total soluble sugars of Khella plant. The increases in TSS might be because of different used treatments stimulated photosynthetic output of plant. These results might increase the efficiency of solar energy conversion which maximized the growth ability of Roselle plant [68]. Different treatments might also be assumed to inhibit polysaccharide-hydrolyzing enzyme system on one hand and accelerate the incorporation of soluble sugars into polysaccharides on another hand. This assumption could be supported by the result that SA increased polysaccharide level that is related to soluble sugars [9]. Concerning the soluble carbohydrate fractions, the application of different treatments led to a marked increase in the galactouronic acid, glucose and rhaminose fractions above the control. [50] isolated distinct sugar fractions from the flower buds of *Hibiscus sabdariffa* L. The fractions were rhaminose, arabinose, galactouronic acid (24%) and glucose [44, 86]. They suggested that these sugars might be involved in decreasing the blood pressure, relaxation of rat uteri, inhibition of taenia motility and bacterial growth in aqueous extract.

Conclusion:

All studied treatments (tryptophan, K, t-cinnamic acid or banana peel extract) especially banana peel extract at 5% concentration increased yield quantity of sepals and quality as of Roselle plant, enhanced good protection under newly reclaimed sandy soil and increased the different secondary metabolites and DPPH radical scavenging activity of Roselle sepals.

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