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Impact of Propolis Extract as Foliar Spray On Growth, Yield and Some Chemical Composition of Spinach (Spinacia Oleracea L.) Plants Grown Under Calcareous Saline Soil

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Abstract

This experiment was suggested to study the beneficial effects of foliar application with propolis extract at the rates 0, 6000, 7000, 8000 and 9000 mg/L solution used as foliar application to spinach seedlings on growth, yield and some chemical constituents of spinach plants (Spinacia oleracea L.) grown under calcareous saline soil conditions. All experiments were achieved during the two successive seasons of 2016 and 2017. Results showed that increasing the rates of propolis extract as foliar application increased the growth parameters of the treated plants. The best result was obtained by the rate (8000 mg/L) as a foliar application in both seasons of the study. The same trend was also observed regarding all studied chemical constituents, i.e. chlorophyll a, b and total carotenoids concentration, anthocyanine, total carbohydrates, total and reducing sugars, total free amino acid, free proline, crude protein, total indoles, total phenols, N, P and K in leaves. Thus, the coincident application of propolis extract at (8000 mg/L) as foliar application ingredient is recommended for improving growth, yield and chemical composition of spinach plants and for overcoming the adverse effect of saline conditions.

Keywords: Spinach Plants (Spinacia Oleracea L.); Propolis Extract; Salinity; Growth; Yield; Chlorophyll; Carotenoids; Anthocyanin; Carbohydrates; Sugars; Free Amino Acid; Indoles; Phenols; Proline.

1. Introduction

Spinach (the Spinacia oleracea, L.), as an annual plant with a short growth cycle, is considered as one of the most common leafy vegetable crop, which belongs to family Chenopodiaceae [1]. Spinach is a highly desirable leafy vegetable with good cooking adaptability, a high nutritive value and many important vitamins and minerals [2]. It is used as cocked during the winter seasons, or as can as well as the frozen product. Although spinach can grow in different climates and in soils of low salinity, increasing soil and/or irrigation water salinities can decrease spinach (Spinacia oleracea L.) yield [3]. A general reduction in growth and yield because of salinity is widely documented [4, 5, 6] on spinach plants that, growth and yield of spinach plants were minimized by increasing soil salinity. Many investigators have conducted studies for up salt tolerance of plants [5, 6, 7]. In recent years, there is a growing interest in natural bio-stimulating substances. Propolis (bee glue) is the generic name for the pitchy substance collected by honeybees (Apis mellifera L.) and found to be effective against a spread of microorganism, bacteria, viruses, and fungi. There is varied literature knowledge of characteristically the chemical composition of propolis [8, 9, 10, 11, 12, 13]. Propolis contains many necessary compounds has been detected to have an effect on the activity of many physiological processes in plants [8, 13]. Amino acids, sugars, bound vitamins (particularly, B-group, C and E), minerals, terpenes, and sesquiterpenes are considered or thought-about to be among these necessary compounds. We are aware that terpenes and sesquiterpenes can be significant compounds important to plant growth processes. Terpenoids are thought-about to be the precursors of the many phytohormones (particularly, gibberellins), that are necessary for plants grown under numerous stresses. The useful impact of propolis extract on growth, yield and chemical constituents of plants was accordingly on several species of plants [14, 15, 16, 7, 17]. Accordingly, the aim of this work was to check the impact of propolis extract as foliar application agent on growth, yield and chemical composition of mature spinach plants under saline conditions of saline calcareous soil and to clarify the role of propolis extract in minimizing the injurious impact of salinity on spinach plants.

2. Materials and Methods

The present investigation was conducted during the two successive seasons 2016 and 2017 in the Experimental Station, Faculty of Agriculture, Fayoum University, Egypt. The physical and chemical properties of the soil were tested by the Soil and Water Department, Faculty of Agriculture, Fayoum University using the standard methods described by Klute [18] and Page et al. [19] and are given in Table1.

Table1. Physical and chemical properties of the soil used before sowing in both seasons.

Properties	**2016**	**2017**
Physical		
Clay%	28.70	28.03
Silt %	22.15	22.90
Sand %	49.15	49.07
Texture grade	Sandy clay loam	Sandy clay loam
Chemical		
Organic matter%	1.17	1.25
pH	7.79	7.71
EC (dS m^{-1})	7.78	7.81
$CaCO_3$ %	8.60	8.54
N %	0.05	0.07
Available nutrients (mg kg^{-1}soil)		
P	18.33	18.55
K	0.35	0. 38
Fe	5.86	5.78
Zn	0.70	0.74
Mn	4.81	4.86

2.1. Preparation of Propolis Extract (PE)

Local raw material of propolis was collected from honeybee colonies of the apiary of Faculty of Agriculture, Fayoum Governorate by scraping hives frames and entrances. Collected samples were mixed together and the active ingredients were extracted by ethyl alcohol 95% [20]. The propolis ethanol mixture was filtered and the alcohol was evaporated under vacuum (30C^{o}) using a rotary evaporator, Buchi model 011. The extract was kept cool in the refrigerator (4C^{o}) until use. Propolis extract was diluted by water to give the final concentration required 6000, 7000, 8000 and 9000 mg/L before use. Seed treatments were carried out by soaking spinach seeds in PE or water for 12 hrs before sowing. Table 2. Groups of substances identified in sample of propolis, Based on Walker and Crane [21]

Group	No. of Substance	Group	No. of Substance
Flavonoids Hydroxy Flavones Hydroxy Flavanones	38 27 11	Terpene and sesquiterpene alcohols and them derivatives	7
Benzoic Acid Derivatives Acids Esters	12 8 4	Sesquiterpene and triterpene hydrocarbons	11
Benzaldehyde Derivatives	2	Aliphatic hydrocarbons	6
Cinnamyl, Cinanamic Acid And Its Derivatives	14	Sterols and steroids hydrocarbons	6
Other Acids And Derivatives	8	Minerals	22
Alcohols, Ketons, Phenols And Heteroaomatic Compounds	12	Sugars	7
Amino Acids	24	Chalcones	2

2.2. Seed Treatment

Seeds of spinach plants (Spinacia oleracea L.) were obtained from Vegetable Research Institute, Ministry of Agriculture, Egypt. Seeds were sown on 1st November, for both seasons in pots (30cm in diameter and 50cm in height) each pot was filled with 20 kg calcareous saline soil. Spinach seeds were sown in each pot. Two weeks after sowing at complete germination, plants were thinned to two plants /pot.

2.3. Pot Experimental

2.3.1. Foliar Spray for Seedling

Seedling used in this study was treated by propolis extract (PE) and grouped under five classes as follows: *Control: seedlings sparing with water.

*PE 6000 mg/L seedlings sparing with propolis extract 6000 mg/L

*PE 7000 mg/L seedlings sparing with propolis extract 7000 mg/L

*PE 8000 mg/L seedlings sparing with propolis extract 8000 mg/L

*PE 9000 mg/L seedlings sparing with propolis extract 9000 mg/L

2.3.2. Fertilization

All spinach plants including control were fertilized with NPK full recommended dose by the Ministry of Agriculture, Egypt. Phosphorous as triple calcium superphosphate (45 - 46%P2O5) at the rate of 75 kg/fed., (1.5g/pot) was mixed with soil before sowing. Nitrogen fertilizer was applied in the form of urea (46%N) at the rate of 100 kg/fed., (2g/pot) and 50kg/fed., of potassium sulphate (48% K2O) (1g/pot). The amount of N and K fertilizers was divided into two equal doses, the first was added after two weeks from sowing and the second was added two weeks later.

2.4. Measurements

2.4.1. Growth Character

At harvest time (50days old plants), samples of each treatment (10 plants) were taken. Plant height (cm), number of leaves/plant, fresh and dry weight of leaves/plant (g) were measured on each plant. Total leaf area (cm2) of each plant was estimated by using an area meter, model Li 3000 from LI-COR, USA., s

2.4.2. Chemical Constituents

At the age of 50 days (in both seasons) samples of fresh leaves were taken for chemical determination i.e. photosynthetic pigments: chlorophyll a, b and carotenoids were extracted from fresh leaves by acetone (80%) then, their concentrations were determined (mg/ 100g fresh weight) according to [22]. Total carbohydrates mg g-1 dry weight was determined colorimetrically according to the method described by [23]. Total and reducing sugars were determined according to [24] and recorded as mg g-1 dry weight. Anthocyanin concentration mg/100g dry weight was determined according to the method described by [25]. Total free amino acids in fresh leaves were determined colorimetrically according to the method described by [26] and recorded as mg g-1 dry weight. Total indoles in fresh leaves were determined colorimetrically according to the method described by [27] and recorded as mg g-1 dry weight. Total soluble phenols in fresh leaves were determined according to [24] and recorded as mg g-1 dry weight. Free proline concentration (mg g-1 dry weight) was determined according to [28]. Nitrogen% and crude protein percentage was determined according to micro Kjeldahl as described by [24] phosphorus % was determined according to [24] potassium was determined by Flame Photometer, Parkin–Elmer model 52 according to [19].

2.4.3. Statistical Analysis

The experiment was in a complete randomized block design with 5 treatments and 6 pots as replicates for each treatment. Results were statistically analyzed using the L.S.D. a probability level of 5% for comparisons [29].

3. Result

3.1. Vegetative Parameters

3.1.1. Effect of Propolis Extract (PE) on Growth Characters

Data in Table (3&4) reported that propolis extract as foliar application affected significantly growth parameters (plant height, number of leaves plant-1, total leaf area/plant and fresh and dry weight of leaves per plant). The results showed that using PE as foliar application caused significant increases in growth parameters during the two studied seasons. All tested growth parameters were gradually increased by increasing propolis extract levels up to 9000 mg/L. The highest increase in yield represented in fresh weight of leaves was obtained by using propolis extract at 8000 mg/L., as a foliar application was (72.61 and 90.94%) in the first and second seasons, respectively in compared with the control (seedling spray with water). The same trend was also observed for plant height, a number of leaves/plant, total leaf area /plant and dry weight of plant leaves especially with propolis extract at 8000 mg/L. treatment in comparison to the control plants. Applying propolis extract at 7000 mg/L. effectively alleviated the adverse effects of soil salinity on yield and its components. The highest increases were 69.63 and 68.10% for plant height, 80.85 and 59.33% for a number of leaves/plant, 24.30 and 24.83 % for total leaf area /plant and 94.70 and 89.42 % for dry weight of plant leaves in the first and second seasons, respectively in compared with the control. Table (3) Effect of propolis extract (PE) as a foliar application on plant height, number of leaves/plant, total leaf area and fresh weight of leaves/plant, of spinach plants in 2016 and 2017 seasons.

Treatments		**Plant height (cm)**		**Number of leaves/plant**		**Total leaf area / plant (cm^2)**		**Fresh weight of leaves/ plant (g)**	
		2016	2017	2016	2017	2016	2017	2016	2017
Foliar spray	**Control (water)**	23.99	22.65	11.53	11.36	150.6	150.6	22.46	23.33
	PE 6000 mg/L	28.15	30.00	14.55	12.99	168.2	168.9	26.25	28.11
	PE 7000 mg/L	38.15	39.58	18.21	17.55	184.1	180.1	35.01	33.77
	PE 8000 mg/L	41.41	43.25	19.70	18.10	187.2	188.0	38.10	39.22
	PE 9000 mg/L	38.10	39.11	17.52	17.98	179.1	177.1	34.18	35.44
L.S.D at 0. 5%		**4.52**	**4.35**	**2.55**	**2.52**	**6.70**	**.426**	**4.35**	**4.54**

Effect of Propolis Extract (PE) on Chemical Constituents

3.2. Leaf Pigments Concentration

Data recorded in Table (4&5) reported that, the concentration of leaf pigments (chlorophyll a, b, total carotenoids and anthocyanin) was significantly increased with propolis extract as foliar application treatments comparing with control plants. The data also show that foliar application with (PE) gave the best result in chlorophyll a, b, total carotenoids and anthocyanin of spinach plants. The maximum increase was obtained with (PE) at 8000 mg/L as foliar application which recorded 31.67 and 36.23% for chlorophyll a, 63.84 and 64.60 % for chlorophyll b, 43.02 and 36.13 % for total carotenoids, 75.93 and 81.36 % for anthocyanin in the first and second seasons respectively compared to the control plants.

Table (4) Effect of propolis extract (PE) as a foliar application on the dry weight of leaves/plant, chlorophyll a & b and total carotenoids concentration of spinach leaves in 2016 and 2017 seasons.

Treatments		**Leaf dry weight plant^{-1} (g)**		**Chlorophyll a mg/100g F.W**		**Chlorophyll b mg/100g F.W**		**Total caroteniods mg100/g F.W**	
		2016	2017	2016	2017	2016	2017	2016	2017
Foliar spray	**Control (water)**	3.02	3.12	115.44	110.10	60.55	60.21	13.90	14.14
	PE 6000 mg/L	4.05	3.98	130.25	130.11	86.09	87.42	15.17	18.35
	PE 7000 mg/L	5.38	5.82	140.25	140.98	94.00	92.88	18.92	17.85
	PE 8000 mg/L	5.88	5.91	152.41	150.00	99.21	99.11	19.88	19.25
	PE 9000 mg/L	5.10	5.25	142.10	145.18	95.25	94.33	18.10	18.21
L.S.D at 0. 5%		**.520**	**0.59**	**7015.**	**15.09**	**9.77**	**9.04**	**3.79**	**3.88**

3.3. Total Carbohydrates, Total Sugars and Reducing Sugars

Data recorded in Table (5) clearly show that, in the two successive season's application of propolis extract at the concentrations of 6000 mg/L. up to 9000 mg/L. significantly increased concentration of total carbohydrates, total sugars and reducing sugars as compared to the control plants. The best result was obtained with (PE) at 8000 mg/L. which recorded 25.31 and 28.26 % increase for total carbohydrate, 23.45 and 22.65% for total sugars and 49.96 and 59.27 % for reducing sugar in the first and second seasons, respectively as compared to the control plants. Table (5) Effect of propolis extract (PE) as a foliar application on anthocyanin, total carbohydrates, total sugars and reducing sugars, of spinach leaves in 2016 and 2017 seasons.

Treatments		Anthocyanin concentration mg/100g D.W		Total carbohydrates mg/g D.W		Total sugars mg/g D.W		Reducing sugars mg/g D.W	
		2016	2017	2016	2017	2016	2017	2016	2017
Foliar spray	**Control (water)**	29.01	28.77	198.25	194.06	80.20	80.00	32.32	30.25
	PE 6000 mg/L	39.19	40.15	233.33	241.21	91.20	86.15	36.00	35.41
	PE 7000 mg/L	49.04	49.10	244.91	248.22	96.25	92.55	42.01	43.07
	PE 8000 mg/L	51.04	52.18	248.44	248.91	99.01	98.12	48.47	48.18
	PE 9000 mg/L	49.19	48.85	247.02	246.12	93.58	93.14	45.50	46.41
L.S.D at 0. 5%		**6.58**	**6.33**	**23.01**	**21.15**	**11.20**	**9.19**	**6.12**	**6.04**

3.4. Total Free Amino Acids, Total Indoles, Total Phenols, Free Proline and Crud Protein

Data in both two studied seasons presented in Table (6 &7) clearly show that in the two studied seasons, all propolis extract treatments significantly increased total free amino acids, total indoles, total phenols, free proline and crude protein in spinach leaves in comparing with the control plants. The maximum increase was obtained when 8000 mg/L. of PE was used as foliar application which resulted in 89.97 and 93.36% for total free amino acids, 67.80 and 68.80 % for total indoles, 84.16 and 62.22 % increase for total phenols, 28.38 and 20.75 % for free proline and 55.28 and 52.28 % for crude protein in the first and second seasons, respectively compared with the control plants.

Table (6) Effect of propolis extract (PE) as a foliar application on total free amino acids, total indoles, total phenols and free proline of spinach leaves in 2016 and 2017 seasons.

Treatments		Total free amino acid mg/g D.W		Total indoles mg/g D.W		Total phenols mg/g D.W		Free proline mg/g D.W	
		2016	2017	2016	2017	2016	2017	2016	2017
Foliar spray	**Control (water)**	20.15	19.28	9.11	8.55	8.02	8.63	1.55	1.60
	PE 6000 mg/L	23.98	25.25	11.11	10.88	10.47	11.11	1.87	1.91
	PE 7000 mg/L	30.10	32.32	11.21	12.33	12.44	13.20	1.90	1.91
	PE 8000 mg/L	38.28	37.28	14.35	14.44	14.77	14.00	1.99	1.98
	PE 9000 mg/L	32.01	31.00	13.00	13.58	13.24	12.86	1.92	1.91

L.S.D at 0. 5%	**2.34**	**2.22**	**1.58**	**1.66**	**2.07**	**2.02**	**0.05**	**0.04**

3.5. Nitrogen, Phosphorus and Potassium Concentrations

Data recorded in Table (7) indicate that, leaves of spinach plants contained a high concentration of nitrogen; phosphorus and potassium due to propolis extract applications condition comparing to control plants. Moreover, these elements were significantly increased with increasing propolis extract rates. The maximum increase was obtained from the application of propolis extract at the rate of 8000 mg/L. as foliar applications were 55.23 and 52.30 % for nitrogen, 40.00 and 41.17 % for phosphorous and 17.30 and 18.30 % for potassium in both seasons respectively as compared to the control plants.

Table (7) Effect of propolis extract (PE) as a foliar application on crude protein, nitrogen, phosphorous and potassium of spinach leaves in 2016 and 2017 seasons.

Treatments		**Crud protein %**		**Nitrogen %**		**Phosphorous %**		**Potassium %**	
		2016	2017	2016	2017	2016	2017	2016	2017
Foliar spray	**Control (water)**	19.68	20.31	3.15	3.25	0.35	0.34	1.56	1.53
	PE 6000 mg/L	23.18	23.37	3.71	3.74	0.42	0.40	1.58	1.59
	PE 7000 mg/L	27.75	28.50	4.44	4.56	0.45	0.44	1.69	1.68
	PE 8000 mg/L	30.56	30.93	4.89	4.95	0.49	0.48	1.83	1.81
	PE 9000 mg/L	27.06	27.62	4.33	4.42	0.42	0.41	1.80	1.82
L.S.D at 0. 5%		**2.04**	**2.06**	**0.60**	**0.62**	**0.02**	**0.03**	**0.03**	**0.03**

4. Discussion

It is clear with the knowledge that soil salinity reduces the various metabolic processes that are liable for traditional plant growth. The adverse result on the syntheses of chlorophyll a, b, carotenoids, anthocyanin, sugars, total free amino acids, proline, N, crude protein and plant auxin concentration that occurred as a result of soil salt stress was reduced by treating the plants with propolis extract. In this connection, Nikolaev [30] and Salama et al [31] reported that the increment in leaf pigments concentration of propolis extract-treated plants might well be attributed to the rise in their hormones, and /or that propolis extract enhances mineral absorption, i.e. (Fe and Mn) required for chlorophyll synthesis, since these parts are found among mineral composition of propolis extract. [32] reported that the hyperbolic level of anthocyanin indicates an index for a decent mechanism of plant resistance towards the changes within the environmental conditions. The rise in total sugars concentration could also be attributed to the sweetening of photosynthesis by the impact of propolis

extract dilated stating from its impact on seed presoaking until completely different stages of plant growth. On the opposite hand, propolis extract would possibly overcome the obligatory NaCl-salt stress via accumulation of sugars. So the increase in total sugars concentration might play a very important role in adjusting the osmotic potential of the protoplasm, a conclusion that is in accordance with results obtained by [33,15]. As well as propolis extract contains some matter like terpenoids [21, 34] from that GA3 is synthesized. The rise in total free amino acids and free proline concentrations of propolis extract-treated plants could also be appreciated for that, these plants might show higher degradation rate of proteins and/ or accumulation of many amino acids ensuing from the inhibition of their incorporation into proteins. The increment in protein concentration in propolis extract-treated plants can be attributed to that, propolis extract contains some B- group vitamins [30]. Since Tayeb [35] recorded that, vitamins of B- group acting as coenzymes, are possess some freelance roles within the biochemical processes of plants. Moreover, Rao et al., [36] proved associated increased protein synthesis with increasing in B- group vitamins accumulation might well be through functioning at the interpretation level of protein synthesis. The increment in total soluble phenols concentration in propolis extract-treated plants may well be attributed to the increment in their total sugars concentration, and/or is also thanks to the rise within the metabolic activity of those plants to synthesize shikimic acid [37]. On the opposite hand the rise in total soluble phenols synthesis in propolis extract-treated plants could indicate that, propolis extract would possibly overcome the obligatory NaCl-salt stress via accumulation of the part that constitutes with different elements (i.e. sugars, proline, and total free amino acids) cellular solutes [38] for sustenance of cells state resulting in maintenance of metabolic activities in these plants. Moreover, the increment in total indoles concentration of leaves of propolis extract- treated plants is also attributed to the rise in their total free amino acids that embrace tryptophan amino acid as a precursor of IAA as shown from results of this study. The rise in macro parts in propolis extract-treated plants is also because of the presence of these parts in propolis extract. In this respect, Walker and Crane [21] listed 149 compounds and twenty-two minerals from totally different samples of propolis. Likewise, as propolis extract contain many helpful mineral elements (i.e. K, Mg, Ca, Cu, Zn, Mn, and Fe) which might compensate presoaked seeds and their developed plants under the conditions of shortage of those mineral elements in carbonate soil, and /or that propolis extract could form a coat around the surface of propolis extract-presoaked seeds and make-do as a block for injurious cations and anions of (free radical) carbonate soil, [34,15]. An increasing in the measured growth characters (plant height etc….) of propolis extract-treated plants could also be attributed to the rise in indoles in these plants (Tables 5 and 6) that might induce an increase in cell division and enlargement [39,40]. Also, propolis extract contains some compounds that enhance or alter plant metabolism resulting in the rise within the leaf area [14], e.g. terpenoids which can induce the vigorous growth and /or enhance plant metabolism resulting in the rise in each fresh and dry weight [34]. Therefore, propolis extract exhibits a compensatory result against the hurtful result

of NaCl-salinity. This suggestion could also be because of that, propolis extract contains terpenoids [21] that have the potential to stimulate plant growth, therefore provide the plants a lucid vigor in growth to resist the adverse impact of NaCl-salinity. The positive impact of propolis extract on seed constituents of the soluble substances before planting could also be attributed to the presence of sugars among the constants of propolis extract [21]and or the rise in œ –amylase activity. The rise in total free amino acids could also be due to the presence of amino acids together with tryptophan among the parts of propolis extract [21] and the, therefore, the vital increase in total free amino acids concentration could also be thanks to that, propolis extract inhibits amino acid incorporation into proteins. Furthermore, the rise in total phenols could also be attributed to the rise in metabolic activity of those tested seeds to synthesize shikimic acid [37] iatrogenic by the propolis extract and /or the rise in sugars concentration in propolis extract treated seeds. These results are typically fully in agreement with those obtained by several investigators, [7, 14, 15, 16, 17] on different plants.

5. Conclusions

Propolis extract as seed soaking application to salt-stressed plants has been shown to reinforce plant salt stress defense responses, to act directly and/or indirectly at rising total plant performances (growth and yields) under salt stress. Thus, propolis might offer an effective strategy to alleviate the adverse effects of salt stress through inflated N-utilization, leading to less harm to spinach growth and its protection from dangerous effects of salt stress. Therefore, propolis extract might act to alleviate the severity of salt stress on spinach plants grown on saline soils.

Conflict of Interest

The authors declare that there is no conflict of interest.

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