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Ameliorative Impact of Propolis Extract Presoaking Treatment Combined with Foliar Spray on Performances of Salt-Stressed Spinach (Spinacia oleracea l.) Plants

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Abstract

Two pot experiments were carried out in two consecutive seasons of 2019 and 2020. The propolis extract was used to soak spinach seeds in 0, 1250, 2500, 3750, 5000 and 6250 mg/L solution and spray seedling with the same concentrations. Effects of propolis extract on growth, yield and some chemical composition changes of spinach (Spinacia oleracea L.) under saline soil conditions were also studied. The results obtained showed that with the seed soaking and foliar application, the rate of propolis extract increased, thereby increasing the growth parameters of the treated plants in the two seasons studied. The best results were obtained through a moderate proportion (3750ppm) as seed soaking and(3750ppm) foliar spray. The same trend was observed for all the chemical components, namely chlorophyll a, b and total carotene concentration**,** anthocyanin, total carbohydrates, total and reducing sugars, total free amino acid, free proline, crude protein, total indoles, total phenols, N, P and K in leaves. In addition, immersing seeds in propolis extract before planting can increase the metabolic activity of seeds by increasing the seed value of total and reducing sugar, total free amino acid, total indoles and total phenols and decreasing the value of total carbohydrate. Therefore, it is recommended to use propolis extract at (3750ppm) as seed soaking and (3750ppm) foliar spray as the same time to improve the growth, yield and chemical composition of spinach plants and to overcome the adverse effect of salt conditions.

Keywords: spinach (Spinacia oleracea L.); propolis extract; salinity; growth; yield, anthocyanin; chlorophyll; carotenoids; sugars; indoles; free amino acid; proline; phenols

Introduction

Spinach is an important leafy green vegetable which contains a large number of biologically active compounds and nutrients. These nutrients are not available in most other vegetables, such as r-coumaric acid derivatives with strong antioxidant activity and glucuronic acid derivatives of flavonoids [1]. It was identified as a salt-sensitive vegetable [2]. Salt stress reduced spinach germination, root elongation, seedling growth, chlorophyll content and photosynthesis, and increased membrane permeability [3]. The growth and yield of spinach plants generally decline due to salt [4, 5] and the growth and yield of spinach plants were minimized due to the increase in soil salt. Many researchers have studied the effect of salt on spinach plants [5, 6, 7].

In recent years, people's interest in natural biological stimulants has increased day by day. Propolis (bee glue) is the general name of the fatty substance collected by bees (Apis mellifera L.) and it has been found to be effectively prevent the spread of microorganism, bacteria, viruses and fungi. Regarding the chemical composition of propolis, there are abundant literatures [8, 9, 10, 11, 12, 13]. Propolis contains many essential compounds which have been found to affect the activity of many physiological processes in plants [8,13]. Amino acids, sugars, combined vitamins (especially, B-group, C and E), minerals, terpenes and sesquiterpenes are considered or considered to be these essential compounds. We know that terpenes and sesquiterpenes may be important compounds for plant growth. Terpenoids are considered to be the precursors of the many plant hormones (especially, gibberellins), and are necessary for plants grown under a variety of stresses. Therefore, the beneficial effect of propolis extract on plant growth, yield and chemical composition are the effects on several plants [14, 15, 16, 17,18,19].

Therefore the purpose of this work is to examine the effect of propolis extract as a seed presoaking combined with foliar spray on growth, yield and chemical composition of mature spinach plants under salt conditions of calcareous saline soil and to clarify the effect of propolis on minimizing the harmful effects of salinity on spinach plants.

1. Materials and Methods:

The present exploration was conducted during the two sequent seasons 2019 and 2020 in the Experimental Station, Faculty of Agriculture, Fayoum University, Egypt. The physical and chemical parcels of the soil were tested by the Soil and Water Department, Faculty of Agriculture, Fayoum University using the standard methodologies described by Klute [20] and Page et al. [21] and are given in Table1.

Properties	2019	2020
Physical		
Clay%	28.15	28.20
Silt %	21.18	22.20
Sand %	49.67	49.60
Texture grade	Sandy clay loam	Sandy clay loam
Chemical		
Organic matter%	1.33	1.30
pH	7.71	7.73
EC (dS m^{-1})	7.83	7.80
$CaCO_3$ %	8.56	8.51
N %	0.05	0.06
Available nutrients (mg kg^{-1}soil)		
P	18.30	19.00
K	0.35	0. 38
Fe	5.50	5.61
Zn	0.82	0.81
Mn	4.88	5.00

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

Preparation of propolis extract (PE):

Expatriate 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 members were yanked by ethyl alcohol 95% [22].Filter the propolis ethanol mixture and use Buchi 011 rotary evaporator to evaporat the alcohol under vaccum (30C°).Keep the extract in the refrigerator (4C°) until it is used. Propolis extract is diluted with water to the desired final concentration required 0, 1250, 2500, 3750, 50000, 6250,7500,10000 and 12500 mg/L before use. Foliar application was done by spraying spinach seedlings with the diluted PE solutions three times at 7 days intervals starting from 14 days from planting. Before sowing, soak the spinach seeds in PE or water for 12 hours for seed treatments

Group	No., substance	Group	No., 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

Table 2. Substance groups identified in propolis samples Based on Walker and Crane [23]

Seed treatment:

The seeds of spinach plants (Spinacia oleracea L.) were obtained from Vegetable Research Institute, Ministry of Agriculture, Egypt. Seeds were sown on November 1, in pots (30cm in diameter and 50cm in height) in both seasons, and each pot was filled with 20 kg calcareous saline soil. Sow spinach seeds in each pot. Two weeks after fully germinated sowing, the plants were thinned out into two plants /pot.

For pot experimental:

The seeds used in this study were soaked in propolis extract (PE) or water for 12 hours, and divided into the following 6 categories:

Control: Soak the seeds in water for 12 hours, and water the seedlings sparing

PE 2500ppm: Soak the seeds in PE 1250 ppm for 12 hours, and PE 1250 ppm the seedlings sparing

PE 5000 ppm: Soak the seeds in PE 2500 ppm for 12 hours, and PE 2500 ppm the seedlings sparing

PE 7500 ppm: Soak the seeds in PE 3750 ppm for 12 hours, and PE 3750 ppm the seedlings sparing

PE 10000 ppm: Soak the seeds in PE 5000 ppm for 12 hours, and PE 5000 ppm the seedlings sparing

PE 12500 ppm: Soak the seeds in PE 6250 ppm for 12 hours, and PE 6250 ppm the seedlings sparing

After soaking for a period of time, the seeds are air-dried on filter paper overnight in the room temperature (25C°) air before sowing. Each treatment contains 6 pots. The normal cultural practices for growing spinach plants have been applied.

For laboratory study:

Seeds used in this part of the study were treated by propolis extract (PE) and grouped under seven classes as follows:
Untreated seeds: Seeds without soaking in neither water nor PE.
Water (12 hours): Seeds soaking for 12 hours in water.
PE 2500 mg/L. (12 hours): Seeds soaking for 12 hours in propolis 2500 mg/L.
PE 5000 mg/L. (12 hours): Seeds soaking for 12 hours in propolis 5000 mg/L
PE 7500 mg/L. (12 hours): Seeds soaking for 12 hours in propolis 7500 mg/L
PE 10000 mg/L. (12 hours): Seeds soaking for 12 hours in propolis 10000 mg/L
PE 12500 mg/L. (12 hours): Seeds soaking for 12 hours in propolis 12500 mg/L
After soaking period, seeds were air-dried overnight under the room temperature (25C°). The dried seeds were ground to fine powder for use in the chemical analysis of seeds.

Fertilization:

All spinach plants including control were fertilized with NPK full recommended dose by Ministry of Agriculture, Egypt. Phosphorous as triple calcium super phosphate (45 - 46%P_2O_5) 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% K_2O) (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 at two weeks later.

Measurements:

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.Use the American LI-COR 3000 area meter to estimate the total leaf area (cm^2) of each plant.

Chemical constituents:

At the age of 50 days (two seasons) fresh leaf samples were colleceted for chemical determination, that is, acetone (80%) was used to extract photosynthetic pigments: chlorophyll a, b and caroteniods were extracted from fresh leaves, and then the concentrations was determined (mg /fresh weight 100g), according to [24]**.** The total carbohydrates mg g^{-1} dry weight was determined colorimetrically according to the method described in [25]. Determine the total sugar and reducing sugar according to [26] and recorded as mg g^{-1} dry weightThe anthocyanin concentration mg/100g dry weight was determined according to the method described in [27]. The total free amino acids in fresh leaves were determined colorimetrically according to the method described in [28] and recorded as mg g^{-1} dry weight. The total indoles in fresh leaves was determined colorimetrically according to the method described in [29] and recorded as mg g^{-1} dry weight. Determine the total soluble phenols in fresh leaves were determined according to [26] and recorded as mg g^{-1} dry weight**.** Determine the free proline concentration (mg g^{-1} dry weight) according to [30]. The nitrogen content and crude protein content were determined according to micro Kjeldahl method as described in [26]; phosphorus content; [26] potassium content; Flame Photometer, measured with Parkin–Elmer model 52 according to [21].

Statistical analysis:

The experiment adopted a completely randomized block design and each treatment was repeated 5 times and 6 pots. The probability level of statistical analysis and comparison of the results using the L.S.D. is 5% [31].

3.Result:

Vegetative parameters:

The effect of propolis extract (PE) on growth characteristics:

The data shown in the table (3&4) show that the application method of propolis extract significantly affects the growth parameters (plant height, number of leaf $plant^{-1}$, total leaf area/plant and fresh weight and dry weight of each plant leaf. The results showed that in the two study seasons pre-soaking seeds and foliar spraying with PE would significantly increase the growth parameters. All growth parameters tested are gradually increased by increasing the level of propolis extract to 3750 mg/L. Seeds immersed in propolis extract at 3750 mg/L obtained the highest increase in yield expressed in terms of fresh leaf weight. Compared with the control, the first and second seasons increased (57.77 and 49.05%) respectively, compared with seeds soaked and sprayed with water. The same trend was also observed for plant height, number of leaves/plants, total leaf area /plant and dry weight of plant leaves especially for seeds pre-soaked and foliar sprayed with 3750 mg/L propolis extract. The treatment is compared to the control plants.
Administer propolis extract at 3750 mg/L. effectively alleviated the adverse effects of soil salinity on yield and its composition. The highest increase is 49.67 and 45.28% of plant height, 55.23 and 50.43% of leaf/plant number, 11.35 and 11.27 % of total leaf /plant area, 57.77 and 49.05 % of fresh weight of leaf/plant and 57.27 and 48.82 of dry weight of leaf/plant. Compared with the control, in the first and second seasons.

Treatments	Plant height (cm)		Number of leaves/plant		Total leaf area / plant (cm^2)		Fresh weight of leaves/ plant (g)	
	2019	2020	2019	2020	2019	2020	2019	2020
Control (water)	24.66	25.55	10.50	11.50	153.3	154.3	18.90	20.67
PE 1250 ppm as Seed soaking+1250 ppm as Foliar spray	29.82	30.59	14.20	14.70	160.8	161.9	22.93	24.06
PE 2500 ppm as Seed soaking+2500 ppm as Foliar spray	31.93	32.82	14.60	15.50	165.7	166.8	23.88	25.74
PE 3750 ppm	36.9	37.1	16.3	17.3	170.	171.	29.8	30.8

as Seed soaking+3750 ppm as Foliar spray	1	2	0	0	7	7	2	1
PE 5000 ppm as Seed soaking+5000 ppm as Foliar spray	36.90	37.12	16.30	17.20	170.7	171.7	29.80	30.80
PE 6250 ppm as Seed soaking+6250 ppm as Foliar spray	36.90	37.11	16.30	17.20	170.6	171.6	29.81	30.80
L.S.D at 0. 5%	**4.35**	**4.62**	**2.64**	**2.84**	**6.33**	**.516**	**4.60**	**4.98**

Table 3: The effect of propolis extract (PE) as seed presoaking combined with foliar spray on plant height, number of leaves/plants, total leaf area and fresh weight of leaves/plant, of spinach plants in 2019 and 2020 seasons.

Effect of propolis extract (PE) on chemical constituents:
Leaf pigments concentration:

Data recorded in Table (4&5) clearly show that, the concentration of leaf pigments (chlorophyll a, b, total caroteniods and anthocyanin) was significantly increased for seed presoaked and foliar spray with propolis extract comparing with control plants. The data also showed that seed pre-soaking and foliar spray (PE) had the best effect on the chlorophyll a, b, total caroteniods and anthocyanin of spinach plants. The maximum increase is obtained by (PE) at 3750 mg/L as seed presoaked, and foliar spray compared with the control. The chlorophyll a were 60.84 and 59.64 %, the chlorophyll b was 24.75 and 33.85 %, the total carotenoids were 53.59 and 60.09 % and the anthocyanin were 60.61 and 61.16 % in the two seasons respectively compared to the control plants.

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**	
	2019	2020	2019	2020	2019	2020	2019	2020
Control (water)	2.74	2.99	92.97	93.96	71.57	72.51	12.39	12.53
PE 1250 ppm as Seed soaking+1250ppm as Foliar spray	3.39	3.56	124.80	125.50	86.44	86.81	16.72	16.81
PE 2500 ppm as Seed soaking+2500 ppm as Foliar spray	3.52	3.81	138.81	139.70	95.76	96.65	18.56	18.69
PE 3750 ppm as Seed soaking+3750 ppm as Foliar spray	4.31	4.45	149.59	150.08	96.23	97.06	19.03	20.06
PE 5000 ppm as Seed soaking+5000ppm as Foliar spray	4.29	4.45	149.54	150.05	96.23	97.05	18.99	20.05
PE 6250 ppm as Seed soaking+6250ppm as Foliar spray	4.28	4.43	149.56	150.03	96.22	97.06	18.95	20.04
L.S.D at 0. 5%	**.660**	**0.65**	**22.16**	**22.37**	**11.64**	**11.86**	**4.17**	**4.19**

Table 4: Effect of propolis extract (PE) as seed presoaking combined with foliar spray on dry weight of leaves/plant, chlorophyll a ***&*** b and total caroteniods concentration of spinach leaves in 2019 and 2020 seasons.

Total carbohydrates, total sugars and reducing sugars:

The data recorded in Table (5) clearly show that, in the two successive seasons, the use concentration of propolis extract is 1250 up to 6250 mg/L. compared with the control plants, the concentration of total carbohydrates, total sugars and reducing sugars increased significantly.
The best result are obtained by seed presoaked and spraying the seeds with (PE) at concentration of 3750 mg/L compared to the control plants, the total carbohydrate in the first and second seasons increased by 29.02 and 29.59 % the total sugars increased by 47.25 and 28.30% and the reducing sugar decreased by 30.07 and 28.15 %.

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**	
	2019	2020	2019	2020	2019	2020	2019	2020
Control (water)	30.98	31.98	186.85	187.85	75.45	76.44	33.98	34.88
PE 1250 ppm as Seed soaking+1250ppm as Foliar spray	39.07	39.76	212.45	213.14	85.84	86.51	36.37	37.96
PE 2500 ppm as Seed soaking+2500ppm as Foliar spray	42.11	43.03	224.95	225.74	90.92	91.78	40.99	41.96
PE 3750 ppm as Seed soaking+3750ppm as Foliar spray	49.76	51.54	241.08	243.45	97.07	98.08	44.20	**44.70**
PE 5000 ppm as Seed	49.75	51.51	241.07	243.44	97.03	97.98	44.19	44.66

soaking+ 5000ppm as Foliar spray								
PE 6250 ppm as Seed soaking+ 6250ppm as Foliar spray	49 .7 6	51 .5 2	24 1.0 7	24 3.4 3	97 .0 6	97 .9 4	44 .1 8	44 .6 5
L.S.D at 0. 5%	**7. 43**	**7.. 95**	**22. 25**	**22. 89**	**10 .1 2**	**10 .1 3**	**6. 04**	**6. 95**

Table 5: Effect of propolis extract (PE) as seed presoaking combined with foliar spray on anthocyanin, total carbohydrates, total sugars and reducing sugars, of spinach leaves in 2019 and 2020 seasons.

Total free amino acids, total indoles, total phenols, free proline and crud protein:

The data in Table (6 &7) clearly indicated that in the study seasons, all propolis extract applications significantly rise the total free amino acids, total indoles, total phenols, free proline and crud protein in leaves compared with control plants.

The maximum increase is obtained when 3750 mg/L. of PE is used in the form of seed pre-soaking and foliar spray, which account increase for 75.07 and 66.04% of total free amino acids, 71.21and 72.07 % of total indoles, 72.76 and 69.83 % of total phenols, 6.59 and 6.45 % of free proline and 38.04 and 38.46 % of crud protein, respectively.

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**	
	2019	2020	2019	2020	2019	2020	2019	2020
Control (water)	16.41	17.82	7.33	7.77	7.82	7.99	1.82	1.86
PE 1250 ppm as Seed soaking+1250ppm as Foliar spray	19.76	20.22	9.42	9.87	10.52	10.54	1.91	1.94
PE 2500 ppm as Seed soaking+2500ppm as Foliar spray	24.32	25.12	11.24	11.93	10.93	11.26	1.93	1.96
PE 3750 ppm as Seed soaking+3750ppm as Foliar spray	28.73	29.59	12.55	13.37	13.51	13.57	1.94	1.98
PE 5000 ppm as Seed soaking+5000ppm as Foliar spray	28.62	29.35	12.54	13.30	13.50	13.51	1.90	1.98
PE 6250 ppm as Seed soaking+6250ppm as Foliar spray	28.61	29.31	12.53	13.28	13.50	13.54	1.93	1.96
L.S.D at 0. 5%	**2.85**	**2.60**	**2.05**	**2.03**	**2.60**	**2.66**	**0.04**	**0.03**

Table 6: The effect of propolis extract (PE) as seed presoaking combined with foliar spray on total free amino acids, total indoles, total phenols and free proline of spinach leaves in 2019 and 2020.

Nitrogen, phosphorus and potassium concentrations:

The data from the two study seasons in Table (7) show that the leaves of spinach plants have a high nitrogen; phosphorus and potassium content compared with control plants due to the application conditions of the propolis extract. In addition, the chemical composition increases significantly as the extraction rate of propolis increases. The maximum increase is obtained by presoaking seeds and foliar spraying with propolis extract at the rate 3750 mg/L compared with control plants, the nitrogen in the two seasons were 36.96 and 36.38 %,the phosphorous was14.56 and 11.53 %, and the potassium was 14.56 and 11.53 % respectively.

Treatments	**Crud protein %**		**Nitrogen %**		**Phosphorous %**		**Potassium %**	
	2019	2020	2019	2020	2019	2020	2019	2020
Control (water)	21.37	21.81	3.42	3.49	0.34	0.35	1.51	1.56
PE 1250 ppm as Seed soaking+1250ppm as Foliar spray	23.00	23.56	3.68	3.77	0.38	0.40	1.60	1.60
PE 2500 ppm as Seed soaking+2500ppm as Foliar spray	25.31	26.10	4.11	4.16	0.40	0.40	1.62	1.63
PE 3750 ppm as Seed soaking+3750ppm as Foliar spray	29.50	30.20	4.65	4.76	0.47	0.48	1.73	1.74
PE 5000 ppm as Seed soaking+5000ppm as Foliar spray	29.40	29.80	4.61	4.74	0.47	0.47	1.67	1.72
PE 6250 ppm as Seed soaking+6250ppm as Foliar spray	29.30	29.70	4.58	4.70	0.45	0.46	1.59	1.71

L.S.D at 0. 5%	**2.04**	**2.06**	**0.67**	**0.65**	**0.03**	**0.02**	**0.04**	**0.03**

Table 7: Effect of propolis extract (PE) presoaking combined with foliar spray on protein, nitrogen, phosphorous and potassium of spinach leaves in 2019 and 2020 seasons.

Chemical composition of seeds after presoaking only:
Effect of (PE) seed presoaking on seed content from total carbohydrate, total sugars and reducing sugars:

The data in Table (8) show that compared with untreated seeds or soaking in water for the same soaking time, soaking spinach seeds in propolis extract at all the concentrations significantly reduces total carbohydrates, while however, total sugars and reducing sugars increased significantly.PE had the highest increase in total sugars in the first and second seasons, which were (29.13 and 33.54 %) respectively, at 7500 mg/L compared to seeds immersed in water. The same trend was observed for reducing sugars. The increase was (61.05 and 63.24 %) at 7500 mg/L. compared with water soaking, PE soaks the seeds separately in the first and second seasons.

Treatments	**Total carbohydrates mg/g D.W of seeds**		**Total sugars mg/g D.W of seeds**		**Reducing sugars mg/g D.W of seeds**	
	2019	2020	2019	2020	2019	2020
Untreated seeds	503.14	506.43	20.92	21.21	4.03	4.11
Control (water)	486.32	489.61	21.16	22.45	4.16	4.19
PE 2500 ppm as Seed soaking	469.99	473.28	25.97	26.26	4.82	4.87
PE 5000 ppm as Seed soaking	459.49	462.78	27.17	28.46	5.37	5.62
PE 7500 ppm as Seed soaking	447.49	450.78	28.99	29.98	6.70	6.84
PE 10000 ppm as Seed soaking	447.42	450.71	28.98	29.97	6.68	6.82
PE 12500 ppm as Seed soaking	447.30	450.50	28.97	29.98	6.69	6.82
L.S.D at 0. 5%	17.82	17.85	3.33	3.32	0.54	0.53

Table 8: The effect of propolis extract (PE) as seed presoaking on the total carbohydrates, total sugars and reducing sugars of spinach seeds in 2019 and 2020 seasons.

Effect of (PE) seed presoaking on seed content from total free amino acids, Total indoles and total soluble phenols:

It is clear from the data in Table (9) that compared with untreated seeds or soaked in water, the concentrations of total free amino acid ,total indoles and total soluble phenols of the tested seeds are significantly improved by the PE soaking at all concentration. Compared with the seeds soaked in water, the total amount of free amino acid in the first and second seasons was the highest, 73.63 and 62.56 %,the total indoles was 18.28 and 14.79 % and the total soluble phenols 54.93 and 49.83 % respectively at 7500 mg/L PE.

Treatments	**Total free amino acid mg/g D.W of seeds**		**Total indoles mg/g D.W of seeds**		**Total phenols mg/g D.W of seeds**	
	2019	2020	2019	2020	2019	2020
Untreated seeds	6.50	6.60	3.71	3.65	2.52	2.60
Control (water)	7.32	7.96	4.43	4.46	3.04	3.01
PE 2500 ppm as Seed soaking	9.43	9.66	4.57	4.60	4.21	4.23
PE 5000 ppm as Seed soaking	11.18	11.89	4.71	4.86	4.35	4.31
PE 7500 ppm as Seed soaking	12.71	12.94	5.24	5.12	4.71	4.51
PE 10000 ppm as Seed soaking	12.69	12.92	5.23	5.12	4.70	4.51
PE 12500 ppm as Seed soaking	12.69	12.91	5.24	5.11	4.71	4.51
L.S.D at 0. 5%	1.72	1.70	0.14	0.13	1.17	1.05

Table 9: Effect of propolis extract (PE) as seed presoaking on total free amino acids, total indoles and total phenols of spinach seeds in 2019 and 2020 seasons.

4. Discussion:

Obviously, soil salt will reduce the various metabolic processes responsible for the growth of traditional plant. By treating plants with propolis, the adverse effects on chlorophyll a, b , carotenoids, anthocyanin, sugars, total free amino acids, proline, N, crude protein and auxin concentration caused by soil salt stress are reduced. In this regard, Nikolaev [32] and Salama et al [33] reported that the increase in leaf pigments concentration of plants treated with propolis extract is probably due to the increase in their hormones, and /or the enhancement of minerals by propolis extract.The absorption of chlorophyll (iron and manganese) required for chlorophyll synthesis, because these parts are present in the mineral components of propolis extract.[32] reported that the hyperbolic level of anthocyanin is an indicator of a decent mechanism of plant resistance to changes within the environmental conditions. The increase in total sugars concentration can also be attributed to the swelling of propolis extract from its presoaking of seeds to the influence of the completely different growth stages of the plant, and the sweetening of photosynthesis. On the other hand, propolis extract may overcome the mandatory NaCl-salt stress through the accumulation of sugars. Therefore, the increase in total sugars concentration may plays a very important role in regulating the osmotic potential of the protoplasm. This conclusion is conclusion with results obtained by [35,15]. Propolis extract also contains some terpene like substances [23, 36], which are synthesized by GA_3. The increase in the concentration of total free amino acids and free proline in plants treated with propolis extract can also be understood as these plants may show higher protein degradation rate and/ or accumulation of many amino acids due to the inhibiting their incorporation into proteins. The increase in protein

concentration in plants treated with propolis extract can be attributed to the fact that the propolis extract contains some B-vitamins [32].Since the record of Tayeb [37], B-vitamins as coenzymes have some freelance roles in the biochemical processes of plants. In addition, Rao et al. [38] proved that the increase in protein synthesis is related to the increase in the accumulation of group B- vitamins, which likely to be achieved acting on the explanatory level of protein synthesis. The increase in total soluble phenols concentration in plants treated with propolis extractis probably due to the increase in total sugars concentration and/or the increase in the metabolic activity of these plants to synthesize shikimic acid [39].

In contrast, the increase in total soluble phenols synthesis in plant treated with propolis extract may indicate that, propolis extract may overcome the mandatory NaCl-salt by accumulating parts composed of different elements (such as sugars, proline and total free amino acids) cellular solutes[40] maintain the cells state, thereby maintaining the metabolic activity of these plants. In addition, the increase in total indoles concentration in the leaves of plants treated with propolis extract- was also attributed to the increase in total free amino acids including tryptophan as amino acid as a precursor of IAA as shown from results of this study. The increase in macroscopic parts in plants treated with propolis extract is also due to the presents of these parts in propolis extract. In this regard, Walker and Crane [23] listed 149 compounds and 22 minerals are listed from comletely different samples of propolis. Similarly,because propolis extract contain many beneficial mineral elements (for example, K, Mg, Ca, Cu, Zn, Mn, and Fe), these elements may compensate for the lack of these mineral elements in carbonate soil.
Soaked seeds and their growing plants, and /or propolis extract can form a film on the surface of propolis extract-presoaked seeds, and act as a block of harmful cations and anions of (free radical) carbonate soil, [36,15]. The increase in growth characteristics (plant height etc…..) of plant treated with propolis extract may also be attributed to the increase in indoles in these plants (Tables 5 and 6) which may lead to cell division and enlargement [41,42].Propolis extract also contains some compounds that enhance or modify plant metabolism, which leads to an increase in leaf area [14]. Terpenoids that can induce vigorous growth of plants and /or enhance plant metabolism, resulting in an increase in fresh weight and dry weight [36]. Therefore, the harmful effect of propolis extract on NaCl salt showed compensatory result. This may be because the propolis extract contains terpenoids [23], which have the potential to stimulate plant growth and therefore provide plants with clear growth ability to resist the adverse impacts of NaCl salinity. The positive impact of propolis extract on the seed composition of the soluble substances before planting can also be attributed to the presence of sugars in the constants of propolis extract [23] and or- increasede in œ –amylase activity. The increase in total free amino acids may also be due to the presence of amino acids and tryptophan propolis extract [23], so the significant increase in total free amino acids concentration in propolis extract may also be due to the inhibits of the incorporation of amino acid into proteins. In addition, the increase in total phenols can also be attributed to the increase in the metabolic activity of seeds to synthesize shikimic acid [39]

through propolis extract and /or the increase in sugars concentration in seeds treated with propolis extract, which increases the metabolic activity of these tested seeds. These results are usually completely consistent, with the results obtained by several researchers [7, 14, 15, 16, 17, 18, 19] on different plants.

5. Conclusions:

Propolis extract used as seed soaking agent in combination with foliar spray (PE) to salt-stressed plants has shown the ability to enhance plant salt-stress defense responses, and directly and/or indirectly act on total plant growth (growth and yield) under salt stress. Therefore, propolis may provide an effective strategy to reduce the adverse effects of salt stress by increasing nitrogen utilization, thereby reducing the harm to the growth of spinach, and protecting spinach from dangerous effects of salt stress. Therefore, propolis extract may help reduce the severity of salt stress on spinach plants grown on saline soil.

Conflict of Interest:

The authors declare that there is no conflict of interest.

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