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# **Use of Some Bio-Stimulants for Improving the Growth** and Nutritional Status of Koroneiki Olive Trees

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Abstract: This investigation was carried out during two successive seasons (2013 and 2014) to study the effect of foliar sprays and soil drench applications of Licorice root extract (LRE) at 0.5, 1 and 1.5%, Cytolan (C) at 1, 2 and 4g/l (as Cytolan Star® commercial product contain 24% seaweed extract) either individually or in combinations compared with the control on the vegetative growth and leaf mineral content of 5-years old olive trees 'Koroneiki 'which grown at the Research Station Farm of National Research Center located in El Nobarya district El-Behera Governorate, Egypt. Applications were applied for three times at May, July and September. Generally, the results of the current investigation revealed that, in both seasons, Licorice root extract followed by Cytolan at the different concentrations significantly enhanced the vegetative growth parameters of 'Koroneiki' olive trees, such as shoot length (cm), number of leaves/shoot, shoots number/branch, leaf area (cm²) and total chlorophyll content in comparison with those of the control treatment. Concerning the method of application, the highest levels for all vegetative characters and leaf mineral contents were obtained with the soil drench technique as compared to the foliar spray method. The interaction treatments between the two extracts and the method of application increased all recorded characters in most cases as compared to the control treatment.

**Key words:** Licorice root extract • Cytolan • Vegetative growth • Nutrition status • Olive trees

## INTRODUCTION

Olive (Olea europaea L.) is one of the oldest agricultural tree crops, which belongs to the Mediterranean region and considered as one of the major fruit trees in the world for the nutritional value, either as a table or oil product, pickling and conserved fruits [1]. In Egypt, olive trees are grown in old valley and new reclaimed area due to its tolerance to relatively high temperature, dry weather as well as its adaptability to high soil salinity level and poor soil characteristics [1].

Spraying the carnation plants with licorice extract (3g/l) led to an increase in plant lengths, stem diameter and increasing the longevity of leaf and cut flowers of carnation [2]. Furthermore, spraying cucumber plants with the same extract increased the leaf area, the number of branches as well as the total content of chlorophyll

pigment in the leaves [3]. Also, Al-Sahaf *et al.* [4] stated that "spraying 2.5g/l licorice extract increased productivity of tomato plants and spraying the onion plants with licorice extract gave the highest leaf area [5]. Licorice is an excellent source of Fe and contains the natural sweetener glycyrrhizin, said to be fifty times sweeter than sucrose [6].

Cytolan containing many minerals and some growth regulators, polyamines and vitamins applied to improve nutritional status, vegetative growth, yield and fruit quality in different plants [7, 8].

The main goal of this investigation was to study the impact of foliar sprays and soil application of Licorice root extract (0.5, 1 and 1.5%) and Cytolan (0.5, 1 and 2%) either individually or in some combinations, at three time intervals, May, July and September on vegetative growth and leaf mineral content of olive trees 'Koroneiki'.

Table 1: Physical and chemical properties of the experimental soil.

Particle size distribution (%)				Available nutrients (Cation)				Available nutrients (Anion)						
Sand	Silt	Clav	Soil Texture	Fc dsm <sup>-1</sup>	PH		 Р %		Ca meg/l		CO <sub>3</sub>	HCO <sub>3</sub>	Cl-	SO <sub>4</sub>
90	5	5	Sandy	1.5	8.2	Trace	0.44	0.57	2.65	2.40	-	3.85	53	55.65

#### MATERIALS AND METHODS

This experiment was carried out at the Research Station Farm of National Research Center at El Nobarya distract El Behera Governorate, Egypt, during 2013 and 2014 seasons on 5-years old olive trees 'Koroneiki 'to examine the effect of licorice root extract and seaweed extract treatments at different concentrations (either individually some or in combinations) on the vegetative growth minerals content. The soil texture grade was sandy and the physical and chemical properties of the experimental soil are presented in Table (1). Drip irrigation system was applied using river Nile water and the trees were planted at 4×5 meters apart. Experimental trees were chosen in comparable size and received the same horticultural practices. The experimental design was randomized complete block design with three replicates. The treatments were applied as a foliar spray or soil drench for three times (May, July and September), 1-Control (water only). 2- Licorice root extract (LRE) at 0.5%. 3- Licorice root extract (LRE) at 1%. 4- Licorice root extract (LRE) at 1.5%. 5- Cytolan (C) at 1g/l. 6- Cytolan (C) at 2g/l, 7- Cytolan (C) at 4g/l, 8- LRE (0.5%) + C (1g/l) 9- LRE (1%) + C (2g/l) and 10- LRE (1.5%) + C (4g/l).

Licorice root powder (5, 10 and 15g) was extracted in one-liter warm water overnight then filtered through clothes mesh to obtain 0.5, 1 and 1.5%. The concentrations of Cytolan (1, 2 and 4g/l) were prepared by completely dissolving defined amount of Cytolan Star® (Opal Company, contains seaweed extract 20%, free amino acids 24%, N5%, P 5% and K 5% W/W) substance in one liter of tap water (Table 2). Each treatment was presented with 2.5 liters/tree as a foliar or soil applications and the foliar application was with a handheld sprayer until runoff. To applied determine shoot length (cm), number of leaves/shoot, leaf area (cm2), number of new shoots/branch for each treatment, a secondary branch/each direction of tree (about 1 cm in basal diameter) was selected and three new shoots per secondary branch were tagged (at the begging of the experiment) to be measured at the end of each season (December) for the above-mentioned measurements.

Leaf area was measured as mentioned by Ahmed and Morsy [9]. Total chlorophyll content (recorded

by using Minolta meter chlorophyll SPAD-502 and expressed as SPAD units. Analysis of nitrogen, phosphorus and potassium were performed by the method of A.O.A.C. [10].

**Statistical Analysis:** The data were statistically analyzed using the least significant difference test (LSD) at 5 % levels of probability [11].

#### RESULTS AND DISCUSSION

# **Vegetative Growth**

**Shoot Length (cm):** Results in Table 2 showed the positive effect of using a foliar spray application, which significantly increased the shoot length and recorded the highest value compared with soil drench application in both seasons.

The foliar treatment of licorice root extract in some combinations with Cytolan improved the shoot length compared with individually treatments, however, the matched values were nearly the same.

Taking into consideration the interaction effects between application method and extracts treatments, the recorded data demonstrated that, there were in significant differences among the two levels of foliar spray of Licorice root extract either individually or in combinations. Meanwhile, the foliar application of LER (1.5%) and C (4g/l) was more effective and significantly increased shoot length (33.92 and 35.07cm) compared with the control treatment (16.01 and 16.67cm) in both seasons.

**Number of Leaves per Shoot:** Shoot length of Koroneiki olive trees 'found to be increased by soil application which achieved the significant higher value than those of foliar spray treatment during 2013 and 2014 seasons (Table 2).

Regardless of the concentrations of the two extracts, soil drench of licorice root and Cytolan enhanced leaves number/shoot followed by the individual treatments of two extracts throughout both seasons of the study.

Concerning the results of the interaction, it was observed that, the number of leaves/shoot was significantly increased by soil application of both LER (0.5%) and C (1g/l) in relative to the control treatment in both seasons.

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Table 2: Effect of foliar and soil applications of licorice root and Cytolan either individually or in combinations on vegetative growth of Koroneiki olive trees during 2013 and 2014 seasons.

	2013 Season		2014 Season			
Treatments	Foliar	Soil	Foliar	Soil		
		Shoot length (cm)				
Control	16.01 hi	13.92 i	16.67 ij	14.25 i		
0.5% LRE*	23.59 def	25.92 cde	25.06 def	26.78 cd		
1% LRE	27.08 bcd	26.50 b-e	28.67 bcd	26.84 cd		
1.5% LRE	30.50 ab	20.17 fgh	31.00 b	21.67 fgh		
1g/l (C) **	24.01 def	20.09 fgh	25.92 de	21.39 fgh		
2g/l (C)	18.92 gh	17.25 hi	20.67 gh	17.75 hij		
4g/l (C)	18.09 hi	18.25 hi	19.67 ghi	19.50 ghi		
0.5% LRE + $1g/l$ (C)	25.17 cde	29.34 bc	26.51 cde	30.22 bc		
1% LRE + 2g/l (C)	27.59 bcd	22.67 efg	28.33 bcd	22.84 efg		
1.5% LRE + 4g/l (C)	33.92 a	25.34 cde	35.07 a	26.92 cd		
Mean	24.49 A	21.95 B	25.76 A	22.82 B		
		Number of leaves/sho				
Control	10.83 i	13.50 gh	11.11 k	14.00 ij		
0.5% LRE*	16.67 b-f	16.67 b-f	17.50 b-f	17.17 c-g		
1% LRE	17.00 b-f	17.50 bcd	18.00 b-f	18.17 b-e		
1.5% LRE	15.5 d-g	14.00 gh	16.84 d-g	14.33 hij		
1g/l (C) **	15.17 efg	18.67 bc	15.84 f-i	19.50 b		
2g/l (C)	17.83 bc	15.00 fg	18.67 bcd	16.50 d-h		
4g/l (C)	14.00 gh	15.33 d-g	15.17 g-j	16.17 e-i		
0.5% LRE + 1g/l (C)	14.00 gii 12.17 hi	22.50 a	13.17 g-j 13.34 j	23.11 a		
1% LRE + 2g/l(C)	16.50 c-f	17.50 bcd	16.89 d-g	18.00 b-f		
1.5% LRE + 4g/l (C)	18.83 b	17.34 b-e	19.17 bc	18.17 b-e		
Mean	10.83 i	13.50 gh	11.11 k	14.00 ij		
Wican	10.05 1	<u>*</u>	11.11 K	14.00 Ij		
Control	3.38 d	Leaf area (cm <sup>2</sup> )	2 40 4	4.06 a.4		
Control 0.5% LRE*		3.97 a-d	3.49 d	4.06 cd		
	4.62 ab	4.02 a-d	4.87 abc	4.51 a-d		
1% LRE	3.83 bcd	4.70 a	4.09 bcd	5.38 a		
1.5% LRE	3.79 cd	3.63 cd	4.22 bcd	3.83 cd		
1g/l (C) **	4.30 abc	4.31 abc	4.49 a-d	4.53 a-d		
2g/l (C)	3.95 a-d	3.44 d	4.12 bcd	3.85 cd		
4g/l (C)	3.78 cd	4.19 a-d	4.11 bcd	4.44 a-d		
0.5% LRE + 1g/l (C)	3.92 a-d	4.33 abc	4.53 a-d	4.52 a-d		
1% LRE + 2g/l (C)	4.04 a-d	4.28 abc	4.46 a-d	4.84 abc		
1.5% LRE + 4g/l (C)	4.38 abc	4.66 a	4.83 abc	5.12 ab		
Mean	3.99 A	4.15 A	4.32 A	4.51 A		
		Number of new shoo				
Control	0.83 fgh	0.67 gh	1.50 efg	1.17 g		
0.5% LRE*	2.83 ab	2.00 b-e	3.33 b	3.00 bcd		
1% LRE	2.67 abc	0.33 h	3.67 ab	1.50 efg		
1.5% LRE	1.50 d-g	2.00 b-e	3.17 bc	2.84 bcd		
1g/l (C) **	0.67 gh	0.50 h	1.67 efg	1.33 fg		
2g/l (C)	1.67 def	2.33 a-d	2.17 def	3.67 ab		
4g/l (C)	3.17 a	2.33 a-d	4.34 a	3.01 bcd		
0.5% LRE + $1g/l$ (C)	1.17 e-h	0.83 fgh	1.83 efg	1.67 efg		
1% LRE + $2g/l$ (C)	0.50 h	1.17 e-h	2.33 cde	2.17 def		
1.5% LRE + 4g/l (C)	1.83 cde	0.33 h	3.00 bcd	1.83 efg		
Mean	1.68 A	1.25 B	2.70 A	2.22 B		

Means followed by the same letter(s) are not significantly different at 5%.

<sup>\*</sup>LRE= licorice root extract \*\*C= Cytolan (20% seaweed extract)

**Leaf Area (cm²):** The obtained results of Table (2) revealed that, the soil application method insignificantly increased leaf area during the 1<sup>st</sup> and 2<sup>nd</sup> seasons in relative to a foliar application method.

Irrespective of the two extracts concentrations, a soil drench of licorice root extract in combination with Cytolan improved leaves number/shoot compared with individually treatments of two extracts throughout both seasons of the study.

As regard to the results of the interaction between application method and extracts treatments, it is appeared that leaf area increased by soil application of licorice root extract (LRE) (1%) as single treatment followed by soil drench of 1.5% licorice root+4g/l Cytolan with significant differences in the 2<sup>nd</sup> season only compared to soil treatment of control tree.

By increasing the levels of licorice root and seaweed extracts either individually, which applied as a foliar spray or soil drench, leaf areas were decreased.

**Number of New Shoots/Branch:** Data presented in Table (2), indicated that, in both seasons, the number of new shoot/branch was significantly and positively affected by applying foliar spray compared with soil application.

Disregarding the concentrations of the two extracts, a foliar spray of licorice root extract individually had the highest value of shoot number/branch compared with Cytolan treatments either individually or in combinations.

The interaction effects indicated that, foliar spray of Cytolan (C at 4g/l) in both seasons recorded higher number of new shoot per branch in comparison with the control treatments.

The significant increase in vegetative growth parameters of 'Koroneiki' olive trees (shoot length, number of leaves, leaf area and number of new shoot/branch) as result of different treatments of licorice root extract compared to control treatment in the present study, may be attributed to its high content of mevalonic acid which is essential for the synthesis of GA3 acid in plants, where cell division and elongation were enhanced (AL-Jawary, 2002) [12]. Potential of Cytolan Star® in this investigation, which provides an excellent source of bioactive compounds such as free amino acids (24%) and seaweed extract (20%) and macro-nutrients (N-P-K) (5%) that may be affect cellular metabolism in treated trees and leading to enhanced vegetative growth in comparison to control trees [13]. The results of our study indicated that, applying Cytolan individually at low rate (1g/l) as either foliar or soil applications was more effect to enhanced shoot length, number of leaves/shoot and leaf area than higher rates. These results confirmed with that obtained by Crouch and Van [14]. There is a significant increase in vegetative growth such as leaf area and the number of branches due to foliar spray of licorice extract compared with control treatment [8]. Algae extract or seaweed extracts led to significant increases in shoot length, the number and area of leaf rather than the check treatment [15].

**Total Chlorophyll Content (SPAD Units):** Data presented in Table (3) showed that the differences between foliar spray and soil applications did not attain the significant level as the matched values were nearly the same in both seasons. Meanwhile, soil drench treatment increased total chlorophyll content in the leaf tissues than the foliar application in both seasons.

Concerning the results of the interaction, it was observed that a progressive increase on total chlorophyll content in the leaves was observed due to increasing concentration of LRE extract applied either as foliar spray or soil drench.

Moreover, during 2013 season, the highest significant increments in total chlorophyll content were dedicated with individual treatment of licorices root extract (1.5%) as foliar followed by soil application of licorice root extract (1.5%)+SWE(4g/l) compared with control testaments. While, during 2<sup>nd</sup> season, foliar as well as soil application of licorice root extract (1.5%)+SWE(4g/l) significantly enhanced total chlorophyll content in comparison to control testaments.

A plausible explanation for a significant increasing total chlorophyll contents as results of applied Cytolan Star® at low rate (1g/l) as soil application compared to control treatments, may be due to the fact it contains about 24% free amino acids and amino acids such as betaines, glycine betaine, aminobutyrate are fundamental ingredients in the process of protein and chlorophyll synthesis [16]. Whereas, it has been indicated that betaine may work as a nitrogen source when provided in low concentration [17].

Licorice root extract treatments increased significantly total chlorophyll content as compared to the control [8]. On other hand, exogenous application of Cytolan increased chlorophyll content [13].

Leaf Mineral Content (NPK): Data in Table (4) cleared that, in both seasons of the study, the two application methods had in significant effects on nitrogen content in the leaves of 'Koroneiki' olive trees and the recorded values were nearly the same.

Table 3: Effect of foliar and soil applications of licorice root and Cytolan either individually or in combinations on leaf total chlorophyll of Koroneiki olive trees during 2013 and 2014 seasons-

	Total chlorophyll content (SPAD units)							
	2013 Season		2014 Season					
Treatments	Foliar	Soil	Foliar	Soil				
Control	65.84 f	68.05 def	66.26 g	68.55 d-g				
0.5% LRE*	74.56 ab	73.30 abc	74.78 ab	74.63 ab				
1% LRE	75.39 a	71.72 a-e	75.84 ab	72.83 a-e				
1.5% LRE	76.78 a	75.67 a	76.01 ab	74.96 ab				
1g/l (C) **	72.67 a-d	74.40 ab	73.14 a-d	74.58 ab				
2g/l (C)	67.01 ef	68.83 c-f	68.12 efg	69.38 c-g				
4g/l (C)	66.75 ef	69.98 b-f	67.40 fg	71.60 b-f				
0.5% LRE + 1g/l (C)	75.25 a	73.57 abc	75.70 ab	73.79 abc				
1% LRE + $2g/l$ (C)	72.38 a-d	73.60 abc	72.94 a-d	73.82 abc				
1.5% LRE + 4g/l (C)	75.31 a	76.24 a	76.79 a	77.41 a				
Mean	72.19 A	72.53 A	72.70 A	73.16 A				

Means followed by the same letter(s) are not significantly different at 5%.

Table 4: Effect of foliar and soil applications of licorice root and Cytolan either individually or in combinations on leaf mineral content (NPK) of Koroneiki olive trees during 2013 and 2014 seasons

	2013 season		2014 season		
Treatments	Foliar	Soil	Foliar	Soil	
		N (%)			
Control	0.70 d	0.70 d	0.75 f	0.80 ef	
0.5% LRE*	1.08 abc	1.20 a	0.93 c-f	1.15 abc	
1% LRE	1.17 ab	0.95 a-d	1.28 a	1.17 ab	
1.5% LRE	1.20 a	1.15 ab	1.13 a-d	1.10 a-d	
1g/l (C) **	0.80 cd	1.18 ab	0.85 ef	0.96 b-f	
2g/l (C)	0.71 d	0.79 cd	0.76 ef	0.90 d-f	
4g/l (C)	0.70 d	0.71 d	0.75 f	0.85 ef	
0.5% LRE + 1g/l (C)	0.80 cd	1.00 a-d	0.96 b-f	0.95 b-f	
1% LRE + 2g/l (C)	0.87 bcd	0.88 a-d	1.10 a-d	0.93c-f	
1.5% LRE + 4g/l (C)	0.98 a-d	0.91 a-d	1.12 a-d	0.98 b-e	
Mean	0.90 A	0.95 A	0.96 A	0.98 A	
		P (%)			
Control	0.363 de	0.313 e	0.371 def	0.334 f	
0.5% LRE*	0.391 a-d	0.440 a	0.387 cde	0.435 abc	
1% LRE	0.393 a-d	0.387 bcd	0.415 b-e	0.387 cde	
1.5% LRE	0.397 a-d	0.438 ab	0.389 b-e	0.434 abc	
1g/l (C) **	0.432 ab	0.441 a	0.438 abc	0.430 abc	
2g/l (C)	0.427 ab	0.426 ab	0.436 abc	0.431 abc	
4g/l (C)	0.420 abc	0.430 ab	0.423 bcd	0.439 abc	
0.5% LRE + 1g/l (C)	0.399 a-d	0.361 de	0.410 b-e	0.390 b-e	
1% LRE + $2g/l$ (C)	0.417 abc	0.360 de	0.440 ab	0.368 ef	
1.5% LRE + 4g/l (C)	0.438 ab	0.369 cd	0.481 a	0.376 def	
Mean	0.408 A	0.397 A	0.419 A	0.402 B	
	K (%)				
Control	0.34 h	0.41 gh	0.37 h	0.39 gh	
0.5% LRE*	0.42 fgh	0.50 def	0.44 fgh	0.53 def	
1% LRE	0.47 efg	0.46 efg	0.56 b-e	0.48 efg	
1.5% LRE	0.48 efg	0.52 cde	0.53 def	0.54 c-f	
1g/l (C) **	0.64 a	0.68 a	0.67 a	0.69 a	
2g/l (C)	0.62 ab	0.60 abc	0.64 abc	0.65 ab	
4g/l (C)	0.59 abc	0.64 a	0.68 a	0.64 abc	
0.5% LRE + 1g/l (C)	0.50def	0.53 b-e	0.53 def	0.50 ef	
1% LRE + 2g/l (C)	0.59 abc	0.50 def	0.63 a-d	0.53 def	
1.5% LRE + 4g/l (C)	0.64 a	0.54 b-e	0.64 abc	0.54 c-f	
Mean	0.53 A	0.54 A	0.57 A	0.55 A	

Means followed by the same letter (s) are not significantly different at 5%.

<sup>\*</sup>LRE= licorice root extract \*\*C= Cytolan (20% seaweed extract)

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Regardless the applied concentration of the two extracts, it was observed that, during 2013 and 2014 seasons, solely treatment of LRE as foliar or soil drench was more effective to enhance N% content compared with Cytolan and control treatments.

Concerning leaf phosphorus content (P%), the results of applying methods of the two extracts as shown in Table (9) indicate that, foliar application increased leaf phosphorus content with insignificant differences compared with soil drench in both seasons.

Considering the main effect due to the methods of application treatments on leaf potassium content (K%), data of (Table 9) indicated that insignificant differences were observed among the methods and no clear trend was detected throughout the first and the second seasons. Licorice root extract treatments increased significantly the leaf mineral content (N, P and K) of olive plants as compared to the control plants [8].

Furthermore, the results of the present study cleared that, applying Cytolan as individually at low rate (1g/l) as either foliar or soil applications was more effect to enhanced NPK content than higher rates (2 and 4g/l). The low levels of NPK content as results of high concentration of solely Cytolan treatments as soil drench may be due to the growth regulators present in Cytolan. Since the higher concentrations of growth regulators retard the growth and nutrient uptake by plants roots [18].

### **CONCLUSION**

It could be concluded that, low concentration of Licorice root extract (LRE) (0.5%) and Cytolan (1g/l) applied three times during the growing season either individually or in some combinations (as a foliar spray or soil drench) were the promising treatments; since it improved most of the vegetative parameters, total chlorophyll content and the leaf mineral content (NPK) as compared to control treatments. Further investigations are recommended to evaluate the effect of Licorice root extract and Cytolan on the productivity of Olive trees and the fruit quality.

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