RESPONSES OF YOUNG OLIVE CV. KORONEIKI PLANTS TREATED WITH AN ORGANIC FERTILIZER

Stavros Sotiropoulos[1*], Christos Chatzissavvidis[2], Ioannis Papadakis[3], Viktor Kavvadias[4], Christos Paschalidis[1], Chrysovalantou Antonopoulou[2], Antonia Koriki[1]

[1]University of Peloponnese, School of Agriculture and Food, Antikalamos, 24100 Kalamata, Greece

[2]Democritus University of Thrace, School of Agricultural and Forestry Sciences, Department of Agricultural Development, 68200 Orestiada, Greece

[3]Agricultural University of Athens, School of Plant Sciences, Department of Plant Production Science, 11855 Attica, Greece

[4]ELGO-DEMETER, Soil Science Institute of Athens, 14123 Lykovryssi, Attica, Greece

Abstract

Two-year-old, self-rooted olive plants (Olea europaea L. cv. Koroneiki) were tested. The potted plants were grown in two types of soil: acidic (AC) and alkaline (AL) and they were fertilized with a liquid organic product containing fulvic and humic acids, following four different schedules:

- *Treatments AC1 & AL1: No application of organic product;*
- *Treatments AC2 & AL2: Application of organic product (1.66 ml/kg soil) at the petal fall stage;*
- *Treatments AC3 & AL3: Application of organic product (1.66 ml/kg soil) at the petal fall and 20 days after the petal fall stage;*
- *Treatments AC4 & AL4: Application of organic product (1.66 ml/kg soil) at the petals fall, 20 and 40 days after the petal fall stage.*

In all the above treatments 0.42 g N/kg soil were applied as granular fertilizer (type 21-0-0; ammonium sulfate for acidic soil and 26-0-0; calcareous nitric ammonium for alkaline soil), at the beginning of the experiment.

The results showed that AL4 treatments significantly amplified the vegetative vigor of olive plants (new shoot length), whereas the organic treatments in the acidic soil led to adverse results. Moreover, the experimental data showed that AL3 treatment resulted in a significant increase in the leaf concentration of chlorophyll a, b and carotenoids. Significant increase in leaf dry weight was also observed in AC4, AC3 and AL3 treatments, at the end of the experiment.

Keywords: *carotenoids, chlorophyll, nutrition, olive fruit, organic fertilizer, vigor*

1. INTRODUCTION

In Greece, the olive tree has been cultivated since ancient times, as evidenced by excavation findings. The origin of the olive tree is lost in the legends and traditions of the peoples around the Mediterranean. And this is natural, since the olive tree is so closely tied, with the history of the Mediterranean peoples, and their rational diet. Olive is the most extensively cultivated oleiferous tree species in the world, covering an area of 10 million of hectares (Yang et al., 2007). Olive oil is a great product of great economic and social importance. Its consumption is responsible for the low frequency of various chronic diseases. The beneficial properties of virgin olive oil have been attributed to the high proportion of unsaturated / saturated fatty acids, but also to some of its secondary components such as phenolic and volatile compounds from which the health benefits of olive oil arise in the Mediterranean diet (Da-Peng Yang et al., 2007). There are many studies on olive fertilization, but in some cases they are controversial. From a practical viewpoint, the fertilization requirements of olive trees depend on soil type, organic matter content, and pH. Organic fertilizers help plants grow, increase yields and improve the quality and commercial value of production while at the same time are

safe for humans and the environment. They contain, depending on the preparation, many ingredients such as phytohormones (auxins, gibberellins, cytokinins), natural growth bio degraders (betaine), substances of high biological value for plants (polysaccharides, amino acids, proteins, vitamins etc.), macro-micro elements (N, P, K, Fe, Zn, etc.), oligosaccharides, etc. Organic fertilizers, when applied to a crop foliarly or on the soil close to the roots, influence its nutrition pathways by either amplifying its nutrition efficiency or its resistance to abiotic stress, or modify the quality characteristics of the plant, mainly by improving the crop's capacity for nutrient uptake or by improving its nutrient use efficiency (Canellas et al., 2002; Canellas et al., 2009; Mora et al., 2012). The aim of this work was to study the effect of a liquid organic product containing fulvic and humic acids, applied on the soil close to the roots on two-year-old, self-rooted olive plants (*Olea europaea* L. cv. Koroneiki) assessed in Messinia district (southern Greece), grown in acidic and alkaline soils. More specifically, the effect of the above product on vegetation, chlorophyll and carotenoid concentration in leaves, as well as on the leaf dry weight was studied.

2. MATERIALS AND METHODS

2.1. Experimental location experimental plan and olive tree orchards

The study was conducted in one year-old, self-rooted olive (*Olea europaea* L. cv. Koroneiki) plants grown in two soil types in an outdoor protected area. The young olive potted plants were growing in two types of soil: acidic (AC) and calcareous-alkaline (AL). The young plants were automatically irrigated with a drip system and the frequency was determined based on the water capacity of the soils. The plants were placed in the campus of the University of Peloponnese in Kalamata, Messinia, Greece (E22°03′44.784′′, latitude N37°03′38.376′′, altitude 0 m). The experiment was carried out during the year 2019, including four fertilization treatments through foliage with a liquid organic product containing fulvic and humic acids, as follows:

- Treatments AC1 & AL1: No application of organic product;
- Treatments AC2 & AL2: Application of organic product (1.66 ml/kg soil) at the petal fall stage;
- Treatments AC3 & AL3: Application of organic product (1.66 ml/kg soil) at the petal fall and 20 days after the petal fall stage;
- Treatments AC4 & AL4: Application of organic product (1.66 ml/kg soil) at the petals fall, 20 and 40 days after the petal fall stage.

In all the above treatments 0.42 g N/kg soil were applied as granular fertilizer (type 21-0-0; ammonium sulfate for acidic soil and 26-0-0; calcareous nitric ammonium for alkaline soil), at the beginning of the experiment (20 January 2019). The organic product contains 26% humic and fulvic acids as well as nutrients (N 0.1%, P_2O_5 0.01%, K_2O 5%, CaO 0.03%, MgO 0.03%, trace elements, organic C 10.5%).

2.2. Laboratory soil and plant analyses

In order to determine the physicochemical parameters of soils, samples were collected, before the application of the fertilizers (Table 1). Leaf samples were collected in 02 August and 10 December 2019, from the middle part of the shoots. At the beginning of December, the terminal shoot length was measured in central current shoots. All laboratory soil analyses were made according to standard methods such as soil texture by Bouyoucos method, total $CaCO_3$ by Bernard method, organic matter by Walkey-Black method, and cation exchange capacity (C.E.C.) by CH_3COONH_4 method (SSSA, 1990; Okalebo et al., 2002). As regards chlorophylls and carotenoids in leaves, they were determined according to Arnon method (Arnon, 1949; Wintermans and De Mots ,1965; Lone, 2018).

2.3 Experimental pattern and statistical analysis

The experimental pattern was completely randomized with four replications (one tree per replication), thus including 16 plants for each soil type and totally 32 olive plants. The statistical analysis includes an analysis of variance (ANOVA) with the Duncan test using the SPSS statistical package.

3. RESULTS AND DISCUSSION

The two soils of the experiment are characterized as medium in texture, acidic (AC) and calcareous-alkaline (AL) with low organic matter content and sufficient levels of C.E.C. (Table 1).

Table 1. Physicochemical properties of acidic (AC) and alkaline (AL) soils of the experiment.

Soil type	Soil texture*	EC (mS/cm)	pH	$CaCO_3$ (%)	Org. matter (%)	Saturation (%)	Bulk density (g/cm^3)	C.E.C. (meq/100 g)
AC	SL	0.100	6.58	0.03	2.00	35	1.28	25.21
AL	SL	0.092	7.90	33.0	1.06	47	1.18	27.08

*CL= Clay loam; SiL= Silty loam; SCL= Sandy clay loam; CL= Clay loam

In general, Table 2 shows that the treatments have a significant effect on shoot length, leaf dry weight, chlorophyll a, chlorophyll b and total chlorophyll concentration in August and leaf carotenoids concentration in August and December. Soil type affects significantly all the examined parameters except leaf dry weight. The interaction between treatments and soil type affected significantly only shoot length and leaf carotenoids concentration in December. The observed interactions of treatments and soil type can be attributed to the effect of treatments on the soil environment and consequently to the availability of nutrients thus affecting vigor (René et. al., 2017).

Table 2. Effect of treatments type soil and interaction Treatments × Soil on all the examined parameters in the experimental olive plants.

Source	Dependent Variable	df	F	Sig.
Treatments	Shoot length	3	7.271	0.001
	Leaf dry weight	3	87.388	0.000
	Chlorophyll a (August)	3	3.136	0.044
	Chlorophyll b (August)	3	6.478	0.002
	Chlorophyll a+b (August)	3	5.672	0.004
	Carotenoids (August)	3	3.710	0.025
	Chlorophyll a (December)	3	0.861	0.475
	Chlorophyll b (December)	3	1.124	0.359
	Chlorophyll a+b (December)	3	0.937	0.438
	Carotenoids (December)	3	10.611	0.000
Soil	Shoot length	1	15.757	0.001
	Leaf dry weight	1	0.248	0.623
	Chlorophyll a (August)	1	15.370	0.001
	Chlorophyll b (August)	1	14.284	0.001
	Chlorophyll a+b (August)	1	15.495	0.001
	Carotenoids (August)	1	11.378	0.003
	Chlorophyll a (December)	1	10.612	0.003
	Chlorophyll b (December)	1	12.772	0.002
	Chlorophyll a+b (December)	1	12.779	0.002
	Carotenoids (December)	1	31.066	0.000
Treatments × Soil	Shoot length	3	5.676	0.004

	Leaf dry weight	3	0.248	0.862
	Chlorophyll a (August)	3	0.611	0.614
	Chlorophyll b (August)	3	0.456	0.715
	Chlorophyll a+b (August)	3	0.295	0.828
	Carotenoids (August)	3	2.234	0.110
	Chlorophyll a (December)	3	0.934	0.440
	Chlorophyll b (December)	3	2.230	0.111
	Chlorophyll a+b (December)	3	1.466	0.249
	Carotenoids (December)	3	8.529	0.000

The results also showed that organic treatments decreased significantly the vegetative growth of plants (new shoot length) in acidic soil, whereas the organic treatment AL4 in the alkaline soil enhanced it (Table 3). In accordance, significantly smaller shoot growth was frequently observed in young olive plants grown in calcareous soil except the case of AL4 treatment. The treatments involving two and three treatments with the organic product significantly increased leaf dry weight in both soil types with maximum effect in AC3 and AL3 (Table 3).

Table 3. Effect of treatments on the shoot length and leaf dry weight in the experimental olive plants.

Treatments	Shoot length (cm)	Leaf dry weight (g)
AC1	47.50a	0.074c
AC2	22.13bcd	0.067d
AC3	24.63bc	0.095a
AC4	31.13b	0.083b
AL1	13.88cd	0.072cd
AL2	9.00d	0.067d
AL3	11.63cd	0.095a
AL4	37.05ab	0.083b

The means in each column followed by the same letters are not significantly different (Duncan, $P<0.05$).

The interaction between treatments and soil types on shoot length are presented in Figure 1a and Table 2. It is worth mentioning the cases of T1×S and T3×S, T1×S and T4×S and T2×S and T4×S. As far as leaf dry weight concerns, there was no interaction between treatments and soil types and the presented changes were only due to treatments (Figure 1b, Table 2).

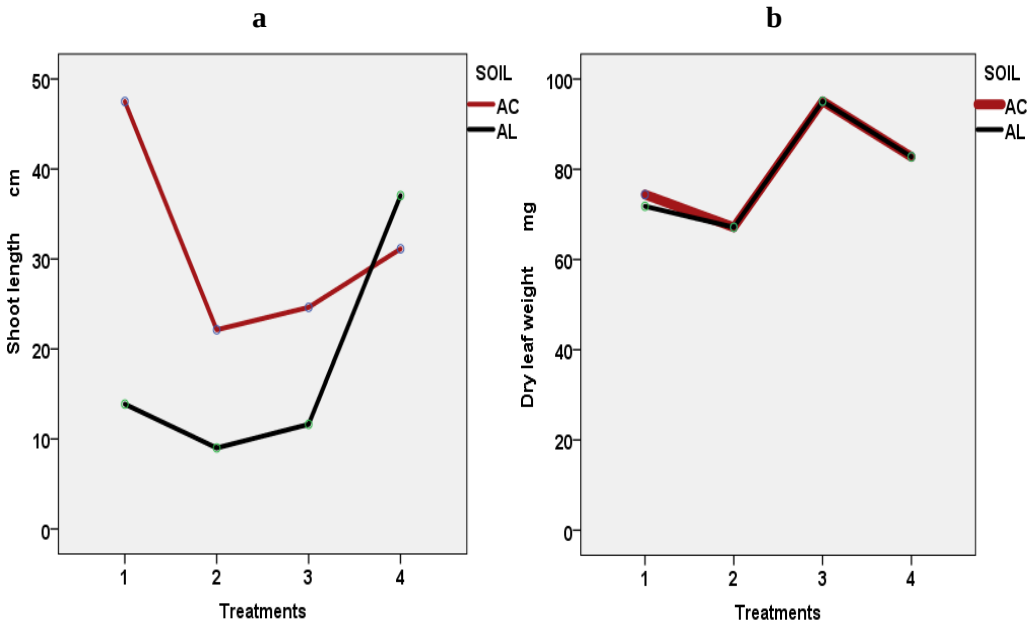


Figure 1. Interaction between treatments and soil types on shoot length (a) and leaf dry weight (b) in the experimental olive plants.

The highest chlorophyll a, b and total chlorophyll concentration was observed in the treatment with two foliar applications (AL3) in the olive plants with alkaline soil in August, however, no significant effect was observed (Table 3). As far the plants in acidic soil, the representative AC3 treatment presented the highest (not significantly) chlorophyll a, b and total chlorophyll concentration. No significant affection was observed in December for the above parameters in both soil types. As expected, the highest values were found in the alkaline soils both in August and December (Tables 4, 5). But, from our macroscopic observations and from the relevant measurements of magnesium and iron concentration in the soils, there is no significant difference between soils in the case of calcium, in contrast to the greater concentration of iron in acidic soil although in both soils they are at the level of adequacy. As it is well known, despite the fact that iron is not a component of chlorophyll, it is considered one of the necessary elements for its formation (Del Rio et al., 1978). The highest ratio of chlorophyll a to chlorophyll b was presented in AC4, both in August and December. The lowest values of both chlorophyll a and b (mainly b) with a significant difference only in chlorophyll b were found in August (Tables 4, 5). A stabilization or decrease in chlorophyll a has been observed over time, where a decrease in chlorophyll b and total chlorophyll concentration at the same time was observed (Tables 4, 5). The highest value of ratio chlorophyll a to chlorophyll b in AC4 during vegetative growth but also, the increase of this ratio during the same period, can be attributed to stress alleviation (Lawlor, 2001; Yang et al., 2009). Regarding the carotenoids concentration in leaves, the highest significant values were recorded in AL2 and AL3 treatments in August, and AL2 treatment in December with the only increase during the vegetative period being recorded in AL2, while in the other treatments small or high reduction was recorded. Given that the concentration of chlorophyll and carotenoids is proportional to photosynthetic activity, the above results could be possibly attributed to the treatments.

Table 4. Effect of treatments on leaf chlorophyll (Chl) and carotenoids (Car) concentration (mg/100 g f.w.) in the experimental olive plants in August.

Treatments	Chl a	Chl b	Chl a+b	Chl a/b	Car
AC1	107.1bcd	231.3b	338.3b	0.465b	9.01bc
AC2	93.8cd	257.8b	351.6b	0.377b	8.50c
AC3	117.5bcd	260.5b	378.0b	0.451b	10.51bc
AC4	77.3d	81.3c	158.6c	1.006a	9.71bc
AL1	134.2abc	307.1ab	441.3ab	0.435b	11.27bc
AL2	161.1ab	337.1ab	498.2ab	0.497b	12.63ab
AL3	176.2a	402.0a	578.0a	0.442b	15.83a
AL4	113.2bcd	233.0b	346.2b	0.496b	9.29bc

The mean values in each column followed by the same letters are not significantly different (Duncan, $P<0.05$).

Table 5. Effect of treatments on leaf chlorophyll and carotenoids concentration (mg/100 g f.w.) in the experimental olive plants in December.

Treatments	Chl a	Chl b	Chl a+b	Chl a/b	Car
AC1	76.7b	44.6bc	121.3b	1.73ab	6.53b
AC2	97.8ab	67.2abc	165.0ab	1.51ab	7.16b
AC3	79.7b	51.0bc	130.6b	1.56ab	6.73b
AC4	78.5b	41.4c	119.9b	2.01a	6.43b
AL1	134.6ab	91.9a	226.6a	1.46ab	9.27b
AL2	115.9ab	66.8abc	182.7ab	1.74ab	19.70a
AL3	100.0ab	74.3ab	174.3ab	1.36b	8.56b
AL4	106.2ab	64.5abc	170.7ab	1.68ab	8.86b

The mean values in each column followed by the same letters are not significantly different (Duncan, $P<0.05$).

The observed significant changes in chlorophyll a, chlorophyll b concentration in August can be attributed to treatments and soil type while the relative changes in December can be attributed only to soil type (Table 2, figure 2). Despite the lack of any significant interaction on chlorophyll b in December, it is noteworthy the case of T2×S.

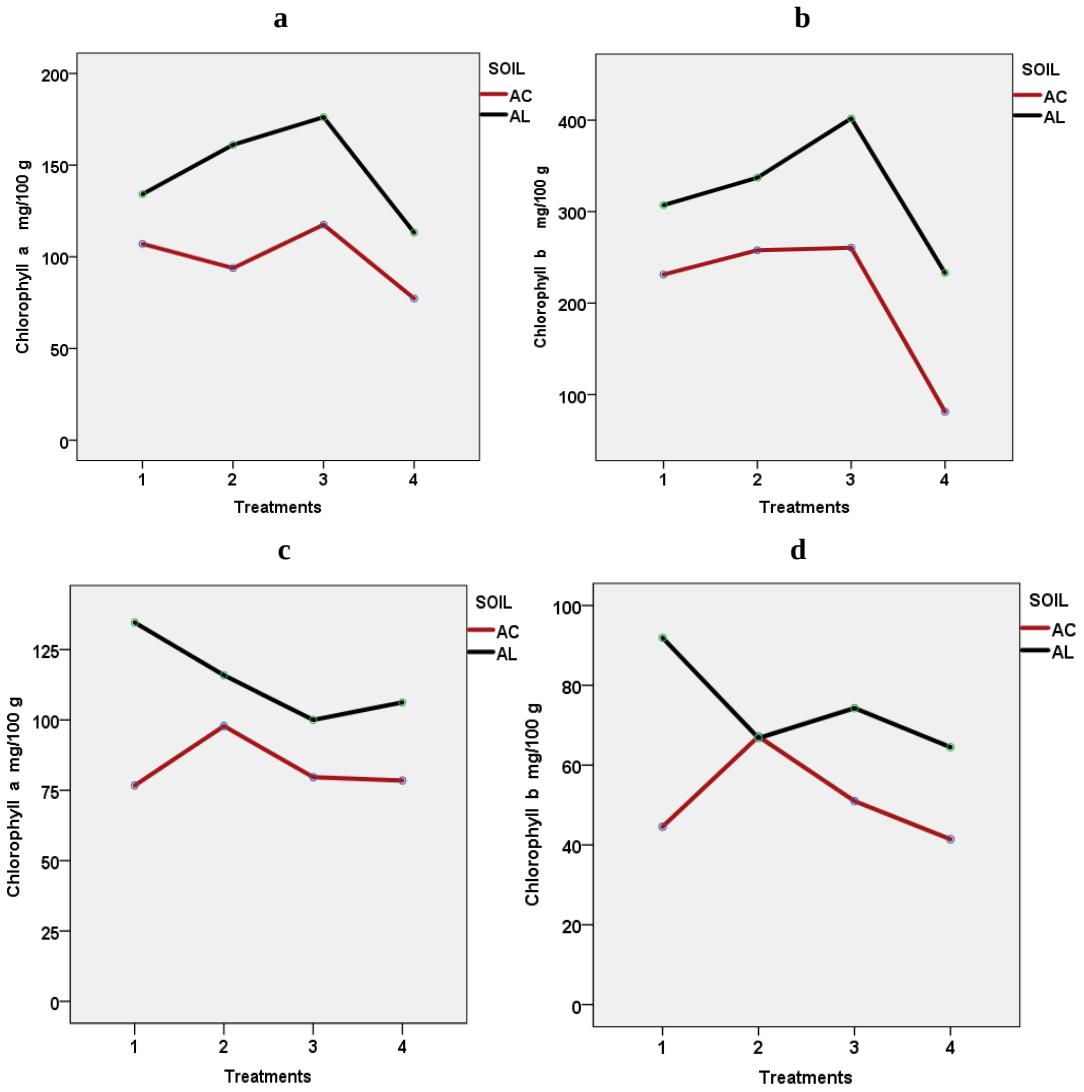


Figure 2. Interaction between treatments and soil types on chlorophyll a and chlorophyll b leaf concentration in August (a, b) and December (c, d) in the experimental olive plants. (Duncan, $P<0.05$)

The significant changes in the chlorophyll a+b are about the same as those of the chlorophyll a and chlorophyll b and can be attributed to the same reasons (Table 2, figure 3a and figure 3c). The significant changes in carotenoid concentrations observed in August and December are due to treatments and soil type and only changes carotenoid concentrations in December are affected from the interaction between soil type and treatments because of S×T2 interaction (Table 2, figure 3b and figure 3d). Despite the absence of significant interactions for chlorophyll b in August, it is worth noting the interactions of S×T3 and S×T4.

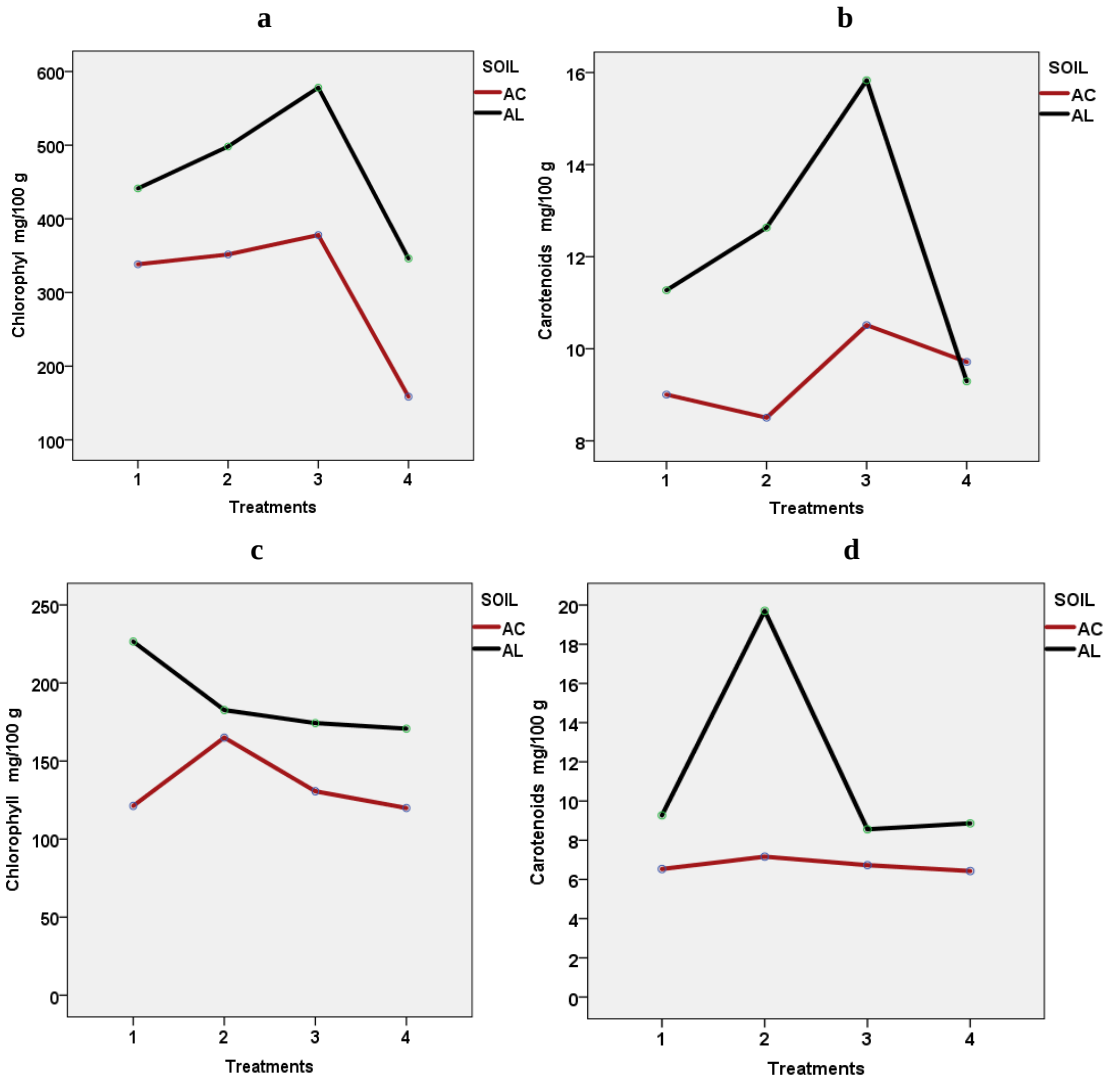


Figure 3. Interaction between treatments and soil types on chlorophyll and carotenoids leaf concentration in August (a, b) and December (c, d) in the experimental olive plants (Duncan, $P<0.05$)

The correlation analysis among leaf chlorophyll concentrations, and carotenoid concentration was conducted to investigate possible relationships among these parameters (Tables 6, 7). Positive correlations were observed among chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentration in leaves in August and December. In line, studies have linked carotenoid regulation to plastid biogenesis and morphology (Lu & Li, 2008). On the other hand, no correlations between chlorophyll a, chlorophyll b, total chlorophyll concentration in August and December with chlorophyll concentration (d.w.) and shoot length were recorded. Positive correlation was found between carotenoid concentration in December and leaf dry weight and shoot length, while the correlation between leaf carotenoid concentration in August with shoot length was negative. Interestingly, there are studies reporting a positive correlation between leaf chlorophyll concentration and shoot growth (Zhao & Takahashi, 1999). Also, a negative correlation was observed between leaf carotenoids concentration in December and leaf dry weight. Finally, no correlation was observed between shoot length and leaf dry weight.

Table 6. Correlation among the examined variables in August, shoot length, olive fruit weight in December

	Chlorophyll a	b	Total a+b	Carotenoids	Shoot length	Leaf dry weight
Chlorophyll a	1	0.888**	0.940**	0.862**	ns	ns
Chlorophyll b	0.888**	1	0.992**	0.789**	ns	ns
Chlorophyll a+b	0.940**	0.992**	1	0.827**	ns	ns
Carotenoids	0.862**	0.789**	0.827**	1	-0.357*	ns
Shoot length	ns	ns	ns	-0.357*	1	ns
Leaf dry weight	ns	ns	ns	ns	ns	1

ns: non significant; **: significant at $P<0.01$

Table 7. Correlation among the examined variables in December

	Chlorophyll a	b	Total a+b	Carotenoids	Shoot length	Leaf dry weight
Chlorophyll a	1	0.845**	0.971**	0.562**	ns	ns
Chlorophyll b	0.845**	1	0.948**	0.460**	ns	ns
Chlorophyll a+b	0.971**	0.948**	1	0.540**	ns	ns
Carotenoids	0.562**	0.460**	0.540**	1	-0.375*	-0.387*
Shoot length	ns	ns	ns	-0.375*	1	ns
Leaf dry weight	ns	ns	ns	-0.387*	ns	1

ns: non significant; **: significant at $P<0.01$

CONCLUSIONS

From the above results, it is clear that the applications with the organic product affected significantly the vigor and the leaf dry weight of the tested plants. The vigor was also affected by the soil type and interaction between soil type and treatments has been found. The chlorophyll concentrations in leaves were affected differently during the growing season whereas the final result is defined by the combined effects of treatments and soil type. The treatments, soil type and their interaction affected the concentration of carotenoids during the growing season. Briefly, and according to the above, interaction of soil type and treatments was recorded only about shoot length and carotenoids, while the soil type affected all the examined parameters except that of leaf dry weight. The above results should be correlated with the nutritional status of the olive tree and the experiment should be continued the following year, in order to obtain a better view of the effect of organic fertilizers applied on young plants grown in different soil types.

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REFERENCES

Arnon DI. 1969. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *B. vulgaris*. Plant Physiology 24: 1-15.

Canellas L.P., Olivares F.L., Okorokova-Façanha A.L., Façanha A.R. 2002. Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H^+-ATPase activity in maize roots. Plant Physiol. 130: 1951–1957.

Canellas L.P., Spaccini R., Piccolo A., Dobbs L.B., Okorokova-Facanha A.L., Santos G.A., Olivares F.L., Facanha A.R. 2009. Relationships between chemical characteristics and root growth promotion of humic acids isolated from Brazilian oxisols. Soil Sci. 174: 611–624.

Da-Peng Y., De-Xin K., Hong-Yu Z. 2007. Multiple pharmacological effects of olive oil phenols. Food Chem. 104: 1269-1271.

Del Rio L.A., Gomez M., Yañez J., Leal A. & Lopez Gorge J. 1978. Iron deficiency in pea plants effect on catalase, peroxidase, chlorophyll and proteins of leaves. Plant Soil 49: 343–353.

Lone J. A., F. A. Lone, M. R. Suseela. 2018. Photosynthetic pigment quantification from two green microalgal species of Scenedesmus. International Journal of Advance Research in Science and Engineering. Volume No. 07, Special Issue No. 04.

Lawlor D.W., 2001. Photosynthesis. Oxford: Biosis Scientific Publishers

Lu S. and Li L. 2008. Invited Review Carotenoid Metabolism: Biosynthesis, Regulation, and Beyond. J. Integr. Plant Biol. 50: 778–785.

Okalebo J.R., Gathua K.W., and Woomer P.L. 2002. Laboratory Methods of Soil and Plant Analysis. A Working Manual. Soil Science Society of East Africa. Technical Publication No. 1.

René R., Marius H., Chistian D. and Prem B., 2017. Effects of Nutrient Antagonism and Synergism on Yield and Fertilizer Use Efficiency. Communications in Soil Science and Plant Analysis.

SSSA (Soil Science Society of America) 1990. Soil Testing and Plant Analysis, Book: Series 3. Wintermans J.F.G.M. and De Mots A. 1965. Spectrophotometric characteristics of chlorophylls a and b and their pheophytins in ethanol. Biochimica et Biophysica Acta 109: 448-453.

Yang D.S., Guo H.X., Wang J.X., 2007. Cultivation and Development of Olive in Sichuan. Sichuan Science and Technology Press, Chengdu.

Yang X., Zhang Z., Joyce D., Huang X., Xu L. and Pang, X., 2009. Characterization of chlorophyll degradation in banana and plantain during ripening at high temperature. Food Chemistry, 114: 383-390.