

EFFECTS OF FOLIAR ORGANIC FERTILIZATION ON 'KORONEIKI' OLIVE TREES IN GREECE

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Abstract

Forty-year-old, self-rooted olive trees (Olea europaea L. cv. Koroneiki) were assessed in Messinia district (southern Greece). Trees were grown in two types of soil: acidic (AC) and alkaline (AL) and they were fertilized through foliage 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: Foliar application of organic product (0.5% v/v) at the petal fall stage; Treatments AC3 & AL3: Foliar application of organic product at the petal fall stage and 20 days after the petal fall stage; Treatments AC4 & AL4: Foliar application of organic product at the petal fall stage, 20 and 40 days after the petal fall stage. In all treatments, 0.8 kg N/ tree were applied as granular fertilizer (type 21-0-0, ammonium sulfate for acidic soil; type 26-0-0, calcareous nitric ammonium for alkaline soil), on 20 January 2019.

The results showed that AC4 treatments significantly enhanced the vegetative vigor of trees (new shoot length), whereas the other organic treatments in the acidic soil did not appear to affect it. Moreover, the experimental data showed that AL3 treatment resulted in an increase (without statistical significance) in the leaf concentration of chlorophyll a, b, whereas AL4 treatment led to a decline in the leaf carotenoids concentration. Significantly increased concentrations of chlorophyll and carotenoids in alkaline soils compared to acidic ones were observed. At the harvest period, olive fruit weight was significantly higher in AC2 and AL2 treatments.

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

1. INTRODUCTION

Olive tree is cultivated almost all over Greece and is native to the Mediterranean region. The olive culture is closely associated with the Western civilization and plays a key role in the Mediterranean 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). The science of nutrition reveals the advantages and the properties of olive, and the medical science verifies its contribution to human health. Beyond its antioxidant potential, many substances are contained in olive oil, 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. In modern agriculture, searching for eco-friendly ways to promote plant growth and enhance crop productivity such as organic fertilizers, is the first priority. Literature concerning the olive fertilization with organic preparations-fertilizers that have recently been released on the market, the results are few and also controversial. Organic fertilizers are formulations which consist mainly of a group of organic substances (such as hormones, humic acids etc.) called bio stimulants, substances from natural origin that contribute to increasing plant yield and nutrient uptake, while reducing the dependency on

chemical fertilizers. Organic fertilizers, when applied to a crop foliarly or on the soil close to the roots, will 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, foliarly applied on forty year-old, self-rooted olive trees (*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 weight of the produced olive fruits was studied.

2. MATERIALS AND METHODS

2.1. Experimental location experimental plan and olive tree orchards

The study was conducted in two olive orchards with forty year-old, self-rooted olive trees (*Olea europaea* L. cv. Koroneiki). The two olive orchards had two types of soil: acidic (AC) and calcareous-alkaline (AL). The tree trunk diameter 15-20 cm above ground was approximately 30 cm on average. The experimental olive orchards are located in the village of Neochorio, prefecture of Messinia in Peloponnese, Greece. The orchard with acidic soil (E21°54'47.022'', latitude N37°02'20.283'', altitude 152 m) had a planting density of 8 m X 8.5 m while the second one with limestone-alkaline soil (E21°54'27.110'', N37°02'10.963'', altitude 178 m) had a planting density of 6 m X 7 m. The experiment was carried out during the year 2019, including four fertilization treatments through foliarage with a liquid organic product containing fulvic and humic acids, as follows:

Treatments AC1 & AL1: No application of organic product;

Treatments AC2 & AL2: Foliar application of organic product (0.5% v/v) at the petal fall stage;

Treatments AC3 & AL3: Foliar application of organic product at the petal fall stage and 20 days after the petal fall stage;

Treatments AC4 & AL4: Foliar application of organic product at the petal fall stage, 20 and 40 days after the petal fall stage.

In all the above treatments, 0.8 kg N/ tree were applied as granular fertilizer (type 21-0-0, ammonium sulfate for acidic soil; type 26-0-0, calcareous nitric ammonium for alkaline soil), on 20 January 2019. Weed control was done by spraying chemicals (glyphosate), without any soil tillage treatment. The organic product contains 26% humic and fulvic acids as well as nutrients (N 0.1%, P₂O₅ 0.01%, K₂O 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 from three depths, 0-30 cm, 30-60 cm and 60-90 cm, before the application of the fertilizers (Table 1). Leaf samples were collected on 02 August and 10 December 2019, from the middle of the shoot. At the beginning of December, during the harvest period, the terminal shoot length was measured in marked samples of current shoots, while at the same time 100 representative olive fruits per tree were collected, from the four cardinal points, and weighed. All laboratory soil analyses were made according to standard methods such as, soil texture by Bouyoucos method, total CaCO₃ by Bernard method, organic matter by Walkey-Black method, and cation exchange capacity (C.E.C.) by CH₃COONH₄ method (SSSA, 1990; Okalebo et al., 2002). As regards chlorophylls and carotenoids in leaves, they were determined according to Arnon method (Arnon, 1949; Winternans 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 trees for each experimental olive orchard, that means totally 32 olive trees. The statistical analysis includes an analysis of variance (ANOVA) with the Duncan test using the SPSS statistical package.

3. RESULTS AND DISCUSSION

The soils of both experimental olive orchards 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 experimental olive orchards.

Depth (cm)	Soil texture*	EC (mS/cm)	pH	CaCO ₃ (%)	Org. Matter (%)	Saturation (%)	Bulk density (g/cm ³)	C.E.C. (meq/100 g)
<i>AC</i>								
0-30	SL	0.100	6.58	0.03	2.00	35	1.28	25.21
30-60	SL	0.282	6.60	0.03	0.80	44	-	28.94
60-90	SCL	0.150	6.42	0.10	0.82	62	-	31.75
<i>AL</i>								
0-30	SL	0.092	7.90	33.0	1.06	47	1.18	27.08
30-60	SC	0.150	8.00	35.7	0.79	60	-	29.88
60-90	C	0.150	7.90	37.5	0.73	65	-	22.41

*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, olive fruit weight, chlorophyll b concentration in August and chlorophyll b in December. Soil type affects significantly all the examined parameters except carotenoids leaf concentration in December. The interaction between treatments and soil type affected significantly chlorophyll a, chlorophyll b, chlorophyll a+b leaf concentration in August and December. Regarding carotenoids, the interaction of treatments and soil type affected the carotenoids leaf concentration only in August.

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

Source	Dependent Variable	df	F	Sig.
Treatments	Shoot length	3	3.060	0.048
	Olive fruit weight	3	10.098	0.000
	Chlorophyll a (August)	3	1.123	0.360
	Chlorophyll b (August)	3	3.294	0.038
	Chlorophyll (August)	3	2.401	0.093
	Carotenoids (August)	3	1.170	0.342
	Chlorophyll a (December)	3	0.771	0.522
	Chlorophyll b (December)	3	3.045	0.048
	Chlorophyll (December)	3	1.132	0.356

	Carotenoids (December)	3	1.636	0.207
Soil	Shoot length	1	7.456	0.012
	Olive fruit weight	1	107.608	0.000
	Chlorophyll a (August)	1	13.619	0.001
	Chlorophyll b (August)	1	26.304	0.000
	Chlorophyll (August)	1	22.864	0.000
	Carotenoids (August)	1	18.728	0.000
	Chlorophyll a (December)	1	33.000	0.000
	Chlorophyll b (December)	1	12.000	0.002
	Chlorophyll (December)	1	26.000	0.000
	Carotenoids (December)	1	0,055	0.816
Treatments X Soil	Shoot length	3	1.681	0.198
	Olive fruit weight	3	0.592	0.626
	Chlorophyll a (August)	3	3.738	0.025
	Chlorophyll b (August)	3	7.831	0.001
	Chlorophyll (August)	3	6.433	0.002
	Carotenoids (August)	3	3.613	0.028
	Chlorophyll a (December)	3	4.757	0,010
	Chlorophyll b (December)	3	9.700	0.000
	Chlorophyll (December)	3	6.530	0.002
	Carotenoids (December)	3	0.431	0.733

The results also showed that AC4 treatment significantly enhanced the vegetative growth of trees (new shoot length), whereas the rest of the organic treatments in the acidic soil did not affect it (Table 3). In alkaline soils, the treatment AL3 had a negative effect, while the rest treatments with the organic product did not affect vigor. In accordance, shorter shoot growth is frequently observed in olive orchards with calcareous soils. This can be connected with poor soil aeration because this increases the concentration of the bicarbonate anion in the soil solution leading in many cases to iron chlorosis, which in our case does not take place both from our macroscopic observations and from the relevant measurements of iron concentration in the leaves. The treatments involving one foliar spraying with the organic product significantly increased fruit weight in both soil types (Table 3). Moreover, in alkaline soil three applications enhanced fruit weight at the harvest period, as well.

Table 3. Effect of treatments on the shoot length and olive fruit weight in the experimental olive orchards.

Treatments	Shoot length (cm)	Olive fruit weight (g)
AC1	8.80bc	1.42bc
AC2	9.80ab	1.68a
AC3	8.38bc	1.53ab
AC4	13.10a	1.54ab
AL1	8.58bc	0.89e
AL2	8.68bc	1.31c

AL3	5.93c	1.05de
AL4	8.05bc	1.09d

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

The figure 1a, shows a positive interaction for shoot length, between treatments and soil types, which is not significantly proven (Table 2). We should have attention to the cases of T1xS and T3xS, T1xS and T4xS and T2xS and T4xS. As far as olive fruit weight, there is no interaction between treatments and soil types (Figure 1b, Table 2) and the presented changes are due to treatments and soil type.

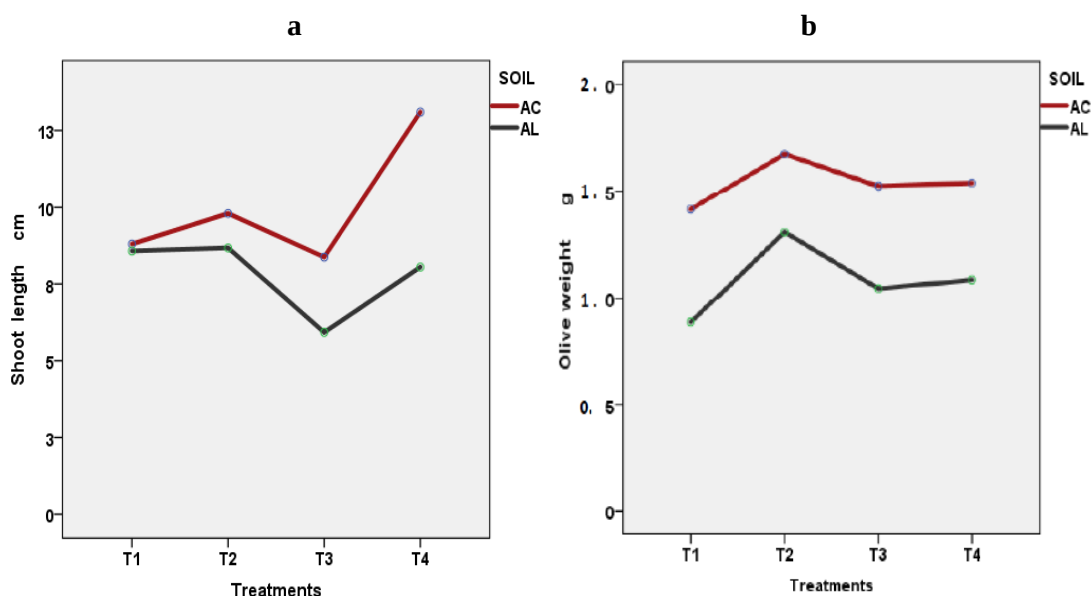


Figure 1. Interaction between treatments and soil types on shoot length (a) and olive fruit weight (b) in the experimental olive orchards.

The highest chlorophyll a concentration was observed in the treatment with two foliar applications (AL3) in the orchard with alkaline soil in August (Table 3), however, no significant effect was observed. The same with significant increase in AC3 and AC4 was observed in December (Tables 4, 5). As regards chlorophyll b concentration in August, AL3 treatment showed a significant increase whereas AL2 treatment led to a significant decline with significant increase in AC3 treatment in December (Tables 4, 5). The highest ratio of chlorophyll a/chlorophyll b occurs in AC3 in August and AC4 in December (Tables 4, 5). An increase in chlorophyll a except AL3 treatment and stabilization or decrease in chlorophyll b have been observed over time, with the exception of AC3 treatment where a high increase in chlorophyll b leaf concentration in December is observed giving the lowest significant chlorophyll a/chlorophyll b ratio and the highest one in August (Tables 4, 5). This reduction can be attributed to the amplified stress in this treatment while, generally, an increase in this ratio is recorded during the growing season (Yang *et al.*, 2009). This may explain the delay in production observed during the growing season (Lawlor, 2001).

Totally, the leaf chlorophyll a+b concentrations in August, presented a significant increase in AL3 treatment, whereas AL2 treatment led to a significant decline. The highest significantly increase in December was presented by AC3, significantly higher than the corresponding treatment in acidic soil AC3 (Tables 4, 5). Given that the concentration of chlorophyll and carotenoids is proportional to photosynthetic activity, it is important to note that is emerged a time lag in the increase in the

concentration of chlorophyll and carotenoids in acidic soil, because of the fact that finally, despite the lower concentrations in August, higher concentrations in mature leaves were observed in acidic soils. The above results, as far as time hysteresis is concerned, could be possibly attributed to the treatments on the trees, and because of the lower levels in soil magnesium content in acidic soil, compared to alkaline soil, taking for granted the sufficient levels of iron in the two experimental orchards. 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). As far as the leaf carotenoids concentration is concerned, no influence was observed except for AL2 and AL4 treatments in August that led to a significant decline whereas no significant influence was observed in December. It is noticeable that no significant changes on the above examined parameters in alkaline soils in December were recorded.

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

Treatments	Chlorophyll			Chlorophyll	Carotenoids
	a	b	Total (a+b)	a/b	
AC1	30.76bc	30	60.85d	1.040bc	3.64b
AC2	33.42bcd	29	63.21cd	1.135ab	3.69b
AC3	30.03bc	23	53.59d	1.298a	3.56b
AC4	34.49bcd	31	65.89abc	1.100bc	4.06b
AL1	38.66ab	39	77.86bc	1.008bc	5.24a
AL2	32.63bcd	27	59.89d	1.205ab	4.28b
AL3	44.03a	49	93.20a	0.899c	5.25a
AL4	37.68abc	41	79.25ab	0.907c	4.00b

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 orchards in December.

Treatments	Chlorophyll			Chlorophyll	Carotenoids
	a	b	Total (a+b)	a/b	
AC1	58.55b	29.20b	87.76bc	2.04bc	4.21a
AC2	59.51b	25.94b	85.45bc	2.32ab	4.02a
AC3	71.30a	48.45a	119.74a	1.49d	3.96a
AC4	71.89a	28.66b	100.55b	2.51a	4.53a
AL1	51.10bc	23.83b	74.94cd	2.19abc	4.01a
AL2	55.63bc	27.58b	83.21bcd	2.02bc	4.46a
AL3	43.01c	20.99b	64.00d	2.05bc	3.88a
AL4	49.00bc	29.47b	78.75cd	1.73cd	4.59a

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 concentration in August can be attributed to soil type and negative interaction between treatments and soil type while the chlorophyll a concentration changes in December can be attributed only to soil type without any interaction influence (Table 2, figure 2a and figure 2c). As far as significant changes in chlorophyll b concentration in August and December can be attributed to treatments, soil type and interaction (negative) between treatments and soil. Only chlorophyll b concentration in December is affected by treatments (Table 2, figure 2b and figure 2d).

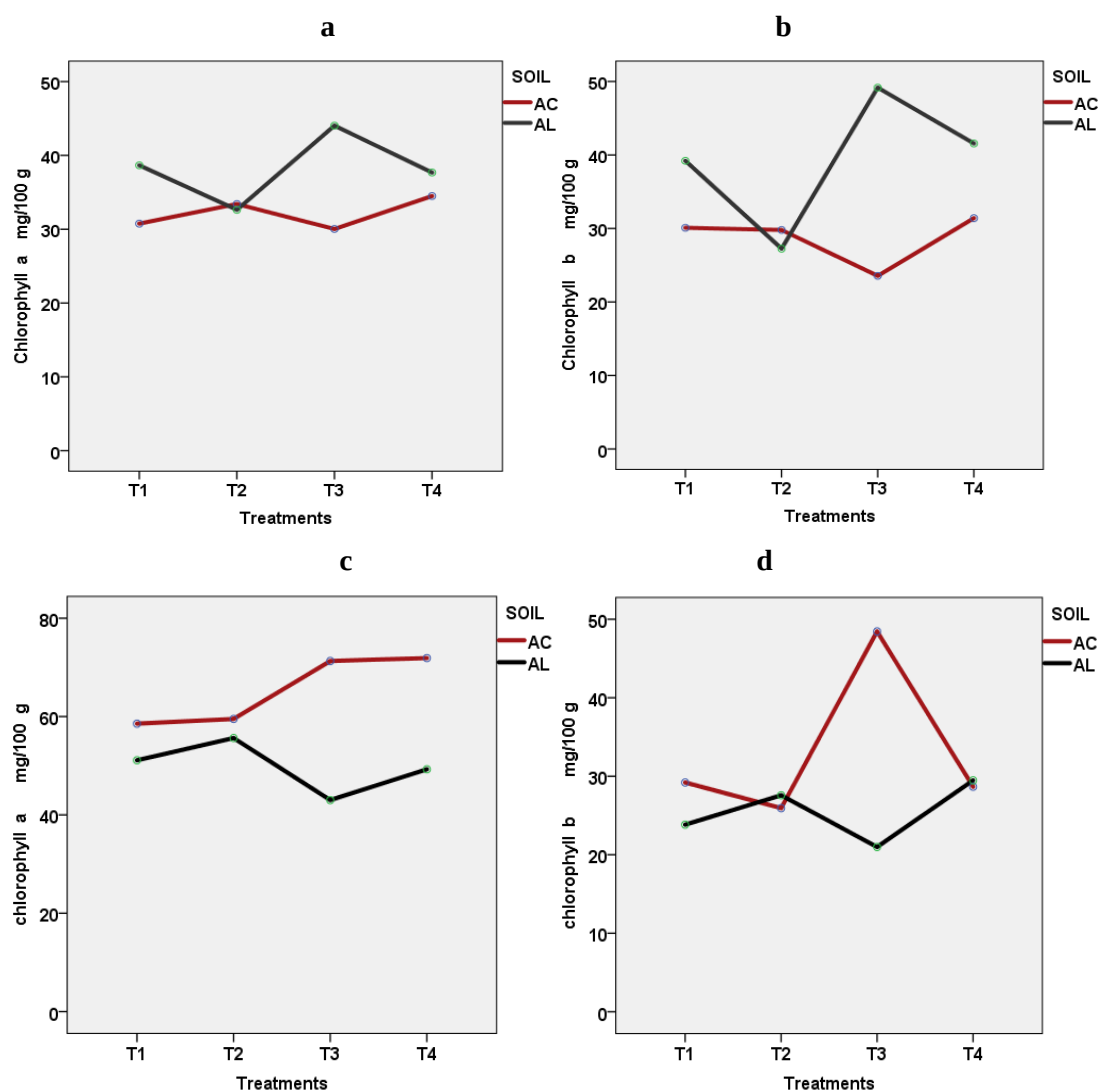


Figure 2. Interaction between treatments and soil types on chlorophyll a and chlorophyll b leaf concentration in August (a, b) and December (c, d) in the experimental olive orchards (Duncan, $P < 0.05$).

The significant changes in the chlorophyll a+b are about the same as those of the chlorophyll b and can be attributed to the same factors (Table 2, figure 3a and figure 3c). The only significant changes in carotenoid concentrations observed in August are due to the negative interaction between treatments and soil type (Table 2, figure 3b and figure 3d).

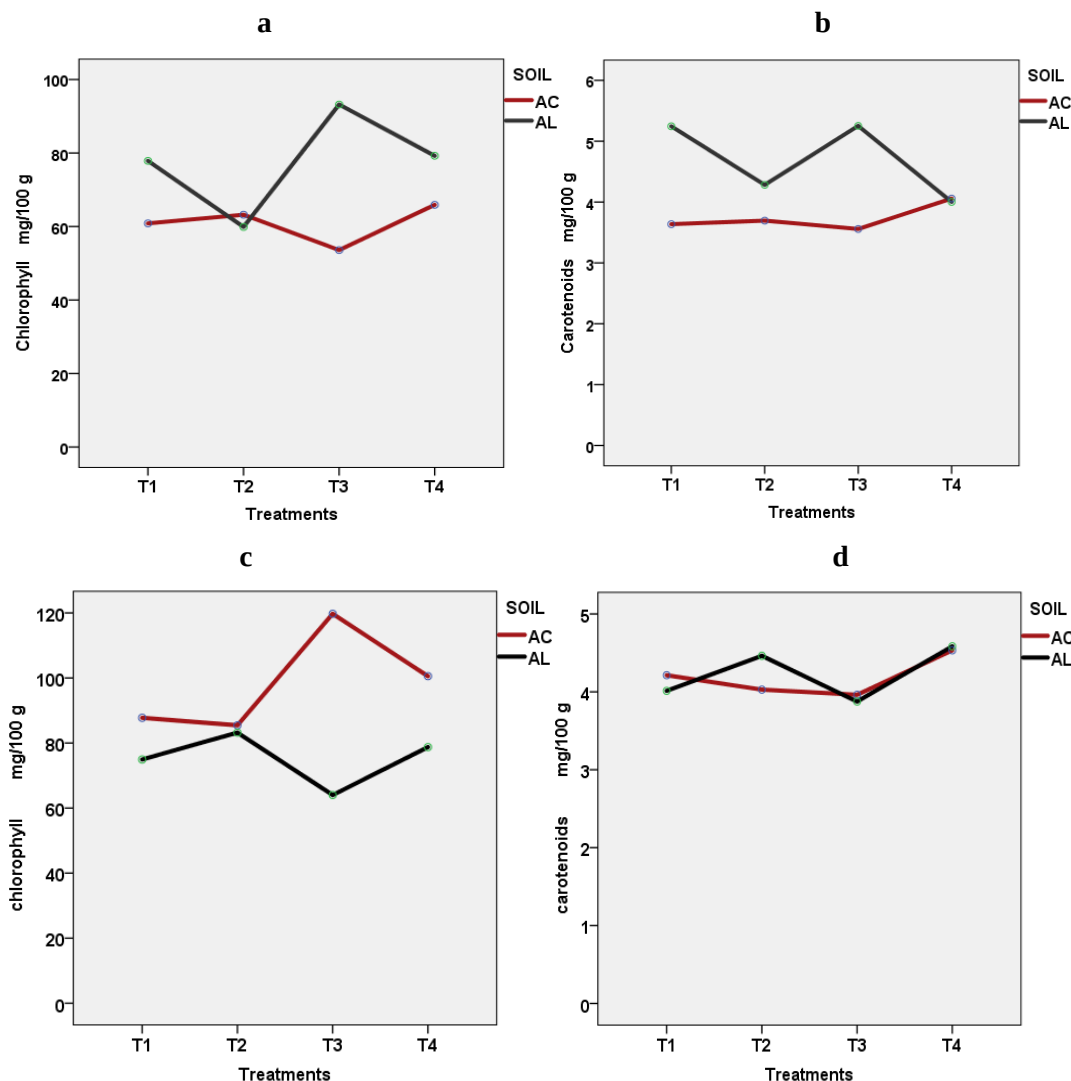


Figure 3. Interaction between treatments and soil types on chlorophyll and carotenoids leaf concentration in August (a,b) and December (c,d) in the experimental olive orchards (Duncan, $P < 0.05$)

The correlation analysis among leaf chlorophyll concentrations, carotenoid concentration, olive fruit weight and shoot length was conducted to investigate possible relationships among these parameters (Tables 6, 7). Highly positive correlations were observed among chlorophyll a, chlorophyll b, total chlorophyll and carotenoid (only in August) concentration in leaves. In line, studies have linked carotenoid regulation to plastid biogenesis and morphology (Lu & Li, 2008). On the other hand, a negative correlation between chlorophyll a, chlorophyll b, carotenoid concentration in August and olive fruit weight were recorded. Exactly the opposite, positive correlation was recorded between chlorophyll a, carotenoid concentration in December and olive fruit weight while with the chlorophyll b and total chlorophyll in December no correlation was recorded. Moreover, there was almost no correlation of the above parameters with the shoot length except with the chlorophyll a concentration in December, whereas there are studies reporting a positive correlation between leaf chlorophyll concentration and shoot growth (Zhao & Takahashi, 1999). Finally, a positive correlation was observed between shoot length and olive fruit weight.

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

	Chlorophyll			Carotenoids	Shoot length	Olive fruit weight
	a	b	a+b			
Chlorophyll a	1	0.875**	0.952**	0.773**	ns	-0.516**
Chlorophyll b	0.875**	1	0.981**	0.734**	ns	-0.620**
Chlorophyll a+b	0.952**	0.981**	1	0.772**	ns	-0.598**
Carotenoids	0.773**	0.734**	0.772**	1	ns	-0.548**
Shoot length	ns	ns	ns	ns	1	0.455**
Olive fruit weight	-0.516**	-0.620**	-0.598**	-0.548**	0.455**	1

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

Table 7. Correlation among the examined variables in December

	Chlorophyll			Carotenoids	Shoot length	Olive fruit weight
	a	b	a+b			
Chlorophyll a	1	0.699**	0.938**	0.423*	0.445*	0.598**
Chlorophyll b	0.699**	1	0.904**	ns	ns	ns
Chlorophyll a+b	0.938**	0.904**	1	0.352*	ns	0.501**
Carotenoids	0.423*	ns	0.352*	1	ns	ns
Shoot length	0.445*	ns	ns	ns	1	0.455**
Olive fruit weight	0.598**	ns	0.450	ns	0.455**	1

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

CONCLUSIONS

From the above, it is clear that the treatments with the organic foliar applications and the soil type affected significantly the vigor and the fruit weight. The concentrations of chlorophylls are affected differently during the growing season where the final result is formed by the composition of the effects of treatments, soil type and their interaction where and when they exist. The treatments and soil type do not affect the concentration of carotenoids while their interaction only affected their concentration in August. 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 better view of the effect of foliar fertilizers applied on trees grown in different soil types.

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