Pak. J. Bot., 52(2): 435-445, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-2(37)

INFLUENCE OF LIGHT INTENSITY AND SOME CHEMICAL COMPOUNDS ON PHYSIOLOGICAL RESPONSES IN OLIVE TRANSPLANTS (*OLEA EUROPAEA* L.)

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Abstract

Leaf physiological responses, chlorophyll content and stomata density of two cultivars Xestawi and Suranni (*Olea europaea* L.) transplants grown under two levels of light intensity (100% and 50%) of full light and different concentrations of calcium and boric acid (0.25% and 0.50%) and (50 and 100 mg.L^{-1}) respectively were studied in addition to control. These studies aimed to discuss the effect of light intensity and some chemical compounds on some physiological responses of olive transplants [net photosynthesis, stomata transpiration, stomata conductance rates and water use efficiency (WUE), in addition chlorophyll *a*, chlorophyll *b*, stomata density and guard cell thickness]. The result showed that all above parameters significantly increased in 100% light intensity except in the case of water use efficiency, it decreased in 100% light intensity. While the effect of mineral compounds on all of the parameters were varied. The transplants treated with 100 mg.L^{-1} boric acid showed higher net photosynthesis, transpiration and stomatal conductance. Hence, higher rates of water use efficiency, stomata density, guard cell thickness and chlorophyll *b* were found in transplants treated with both concentrations of calcium (0.25% and 0.50%).

Key words: Olives, Light intensity, Boric acid, Calcium, Photosynthesis, Transpiration, Stomata Conductance and chlorophyll content.

Introduction

Olive (*Olea europaea* L.) belongs to Oleaceae family, this family includes approximately 30 genera with 600 species. *Olea europaea* L. is an evergreen tree. Since ancient times, olive trees have been cultivated in the Mediterranean region. Olive is a small evergreen tree that can grow between 8-15m in height and is considered to be one of the earliest cultivated plants. They grow very slow and are extremely long-lived species (Rhizopoulou, 2007). Two main products of olive are: oil and table (edible) olives (Sibbett *et al*., 2005). Olive has been considered as a healthy, medicinal and useful plant for all living organisms. Olive oil contains (14.8%) saturated fat and (85.2%) unsaturated fat (Vaughan & Geissler, 1999) and it is valued as an important item for diet (Roche *et al*., 2000). The leaves are used as herbal tea due to presence of phenolic compound (Breton, *et al*., 2012).Virgin olive oil is known for its delicate and unique pleasant smell (Dhifi *et al*., 2005). The olive wood is heavy and very tough normally used for high-end furniture, inlays, turned objects and handcraft. It is also appreciated as firewood because it burns even when wet (Breton *et al*., 2009). In Iraq, olive trees are densely found to be grown in some areas of Kurdistan Region and central Northern Iraq. Nineveh is the leading governorate olive producer. They are heavily cultivated in Nineveh province where some villages are well-known for their olive fields such as: Baashiqa, Bahzany, Fadiliya, Sheikh Uday, Dhecan, Aqra, and Sinjar. Other areas where olive trees are also grown include Babylon, Deyaa, Kirkuk, Baghdad, Erbil and Duhok (Abdul-Qader, 2012). Solar radiation is the main and most significant environmental factor that regulates the photosynthesis on which the plant survival, growth and adaptation depends. The majority of plant species have the ability to develop anatomical, morphological, physiological and biochemical adaptations in response to different light intensities (De Carvalho Gonçalves *et al*., 2005). Olive trees (*Olea europaea* L.) are able to tolerate a broad range of environmental stresses and this capability is due to a variety of morphological and physiological adaptations (Bacelar *et al*., 2004). It is obvious that photosynthesis occurs in plant leaves which determines plant growth and development by converting atmospheric carbon dioxide into carbohydrates. In addition to green leaves, photosynthetic assimilation has also been measured in petioles, green flowers, stems and fruits. (Aschan & Pfanz, 2003). Calcium has a big role in strengthening the cell wall. It is obvious that calcium deficiency levels lead to the damage of the cell membrane and eventually the cells become leaky. As a result, the cell membrane loses its compounds and finally leads to the death of the cell and plant tissues (Marschner, 1995). It is also believed that calcium has an influence on the development of heat shock proteins to help the plant tolerate the stress of prolonged heat (Chang *et al*., 2006). While Boron has a well-established relation to primary cell wall because it can settle down the cell wall matrix by cross-linking of two pectic polysaccharide by a borate bridge (O'Neill *et al*., 2004).In addition to strengthening and development of cell wall, it plays a role in cell division, fruit and seed development, sugar transport and hormone development. It also regulates the action of certain enzymes and auxins (Wojcik & Wojcik, 2006).The different concentration of calcium chloride (5 and 10 mM) pre-treatment groups exhibited noticeably increased chlorophyll content (Arshi *et al*., 2006). Also the (10 mM) calcium chloride pre-treatment group demonstrated increases in chlorophyll content values. While the change in net photosynthesis for the same

concentration of calcium chloride treatment group exhibited a similar trend. Mukhopadhyay *et al*., (2013) investigated the effect of boron deficiency on photosynthesis and antioxidant responses of young Tea (*Camellia sinensis* L.). Young plantlets were treated with boron at (0, 2.5, and 5 µM) for 8 weeks. Following results were found: Boron (0 µM) decreased the photosynthetic rate, stomatal conductance, and transpiration alongside chlorophyll *a*, chlorophyll *b* and carotenoids. On the other hand, plants exhibited to 2.5 µM boron, photosynthetic rate increased in (74%) as compared with boron (0 µM) plants. Also application of boron (5 µM) induced rise (39 and 51%) in stomatal conductance and transpiration respectively in comparison with boron (0 µM) plants. The content of total chlorophyll, chlorophyll *a*, chlorophyll *b*, reduced under boron (0 µM). Beyaz *et al*., (2018) studied the effect of boron on the morphological and physiological responses of sunflower Seedlings by using different levels of boron (0 or control, 0.5, 1.0, 1.5 and 2.0 $mg.L^{-1}$) per pots. The largest leaf areas and chlorophyll content were observed in the seedlings irrigated with water containing (1 $mg.L^{-1}$) of boron compared with the control; while, the highest boron concentration (2 $mg.L^{-1}$) resulted in leaf area being decreased. On the other hand, chlorophyll *a*, chlorophyll *b* and total chlorophyll were decreased with increasing boron. Gregoriou *et al*., (2007) studied the effects of reduced irradiance on leaf morphology, photosynthetic capacity, and fruit yield in olive (*Olea europaea* L.) cv. *koroneiki* grown in plastic containers under full daylight (100%) and (30%, 60%, and 90 %) shade for two years. The results of higher level of shade (30, 60, and 90 %) caused the lower rate of the stomatal and trichomes density, stomatal conductance and net photosynthetic rate. The leaf area was increased about (81 %) in (90 %) shade. Shade levels (30, 60, and 90) % reduced stomatal density by (7, 16, and 27) %, respectively. It also significantly reduced both stomatal conductance, net photosynthesis and leaf mass per area; while, chlorophyll content increased under increasing shade in all seasons of this study. Sofo *et al*., (2009) demonstrated that olive tree (*Olea europaea* L.) of both cultivars (Coratina and Biancolilla) were influenced by the different light level (fall sunlight and semi-shade conditions about 67% of sun light). The high irradiance levels caused a reduction of gas exchange and photosynthetic efficiency.

The objectives of the present study were to focus on the effect of some environmental factors on plants grown in Kurdistan region. The present study aimed to examine the effect of calcium, boric acid and light intensity on some physiological features of two olive cultivars (Xestawi and Suranni) transplants. Producing a large number of olive transplants with good quality and suitable for planting in the permanent places within a short period of time, and creating commercial private olive orchards.

Material and Methods

Young seedlings of *Olea europaea* of two cultivars (Xestawi and Suranni) the transplants were two years and height (30-40 cm) were obtained from a commercial nursery (Alind Nursery). They were transferred to an experimental site of Malta Nursery / Duhok/Iraq and transplanted into 160 polyethylene pots (30 x 30 cm) filled with mixed soil. Then the seedlings were divided at random into two groups which were exposed to two different light intensities. Half of transplants (80 pots) were grown under 50% light intensity (greenhouse) and the rest of transplants (80 pots) were placed under 100% light intensity (direct sunlight) for an acclimation period. Each group was divided into 5 blocks (16 seedlings for each block). The transplants were irrigated at regular periods and all blocks were treated with [control or untreated, calcium (0.25% and 0.50%), and boric acid ($50mg.L^{-1}$ and 100 $mg.L^{-1}$)]. The transplants were grown in a nursery from March until December 2016. Gas exchange measurements, selected from each cultivar and each treatment block, were registered randomly using a Portable Gas Exchange Fluorescence System GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany). All measurements were conducted at a leaf temperature of (30°C). A light response curve for each individual plant was obtained using measurements taken in the dark and under gradually increasing Photosynthetic Photon Flux Density (PPFD) up to (2000 µmol) photons ($m^{-2}s^{-1}$) for sunlight and (1000 µmol photons $m^{-2}s^{-1}$) for shade, (398 ppm) ambient CO_2 and a cuvette relative humidity of approximately (50%). Measurements were taken at 9:00 a.m. (mid-morning) until 16:00 p.m. (mid-afternoon). Vapor Pressure Deficit (VPD) between leaf and air was (25 Pa/kPa) for sunlight and (23 Pa/kPa) for shade. Net photosynthesis, transpiration, water use efficiency (WUE) and stomata conductance rates were measured after the steady state was reached (Kocaçınar, 2015).

Design and analysis of the experiment: The experiment was conducted using a factorial experiment with Randomized Complete Block Design (RCBD) with three factors [2 cultivars × 2 levels of light intensity × 5 concentrations of chemical treatments (calcium chloride and boric acid). Each treatment block included 4 replicates. All data was tabulated and statistically analysed using SAS program (SAS, 2000). The differences between various treatment means were tested with Duncan Multiple Range test at 5% level (Duncan, 1955).

Results

Net photosynthesis: Fig. (1) shows that cv. Suranni gave the highest net photosynthesis as compared with cv. Xestawi during study. From the net photosynthesis, when olive transplants were exposed to different level of light intensity, it was observed the transplants, put under 100% light intensity, showed significant increase in net photosynthesis in comparison with 50% light intensity. On the other hand the maximum net photosynthesis came from transplants sprayed with 0.50% calcium and 100 $mg.L^{-1}$ boric acid, while the minimum net photosynthesis came from 0.25% calcium application.

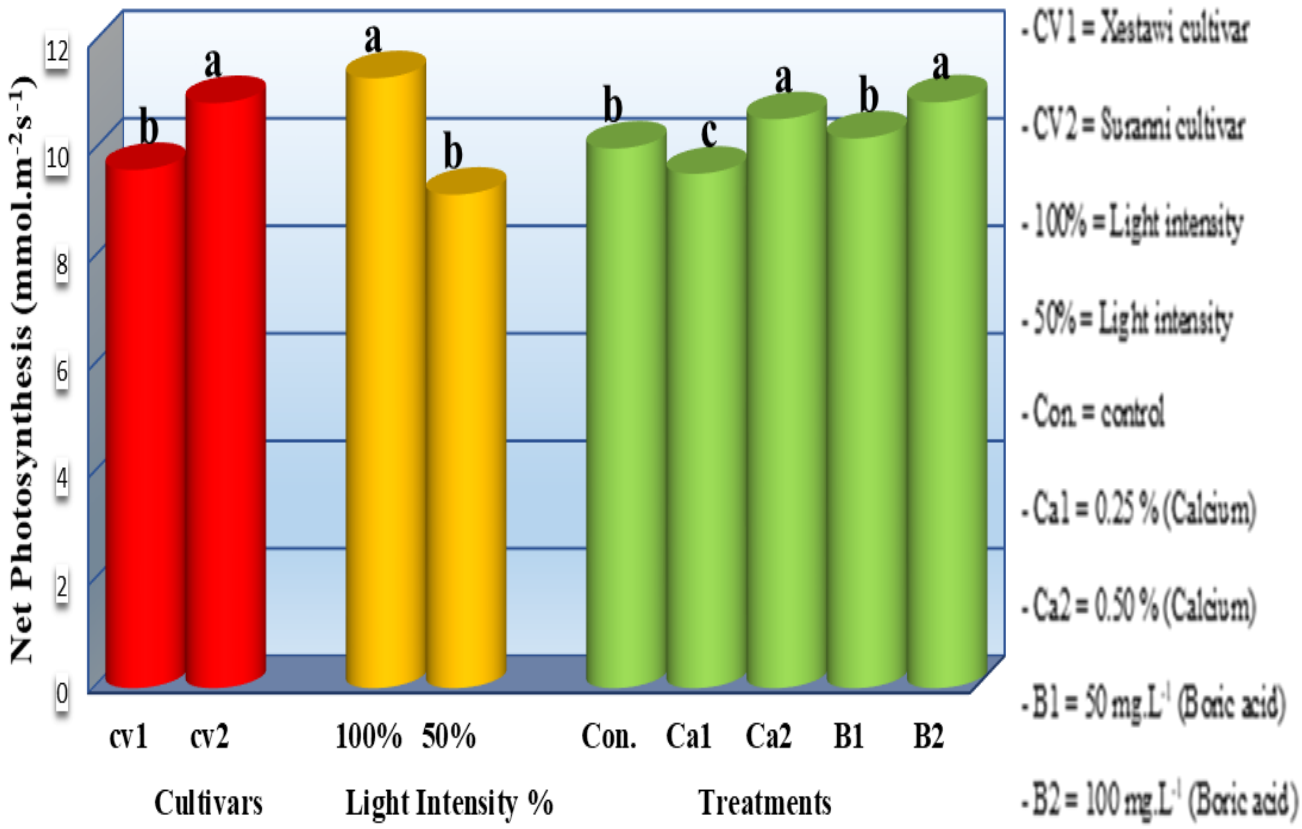

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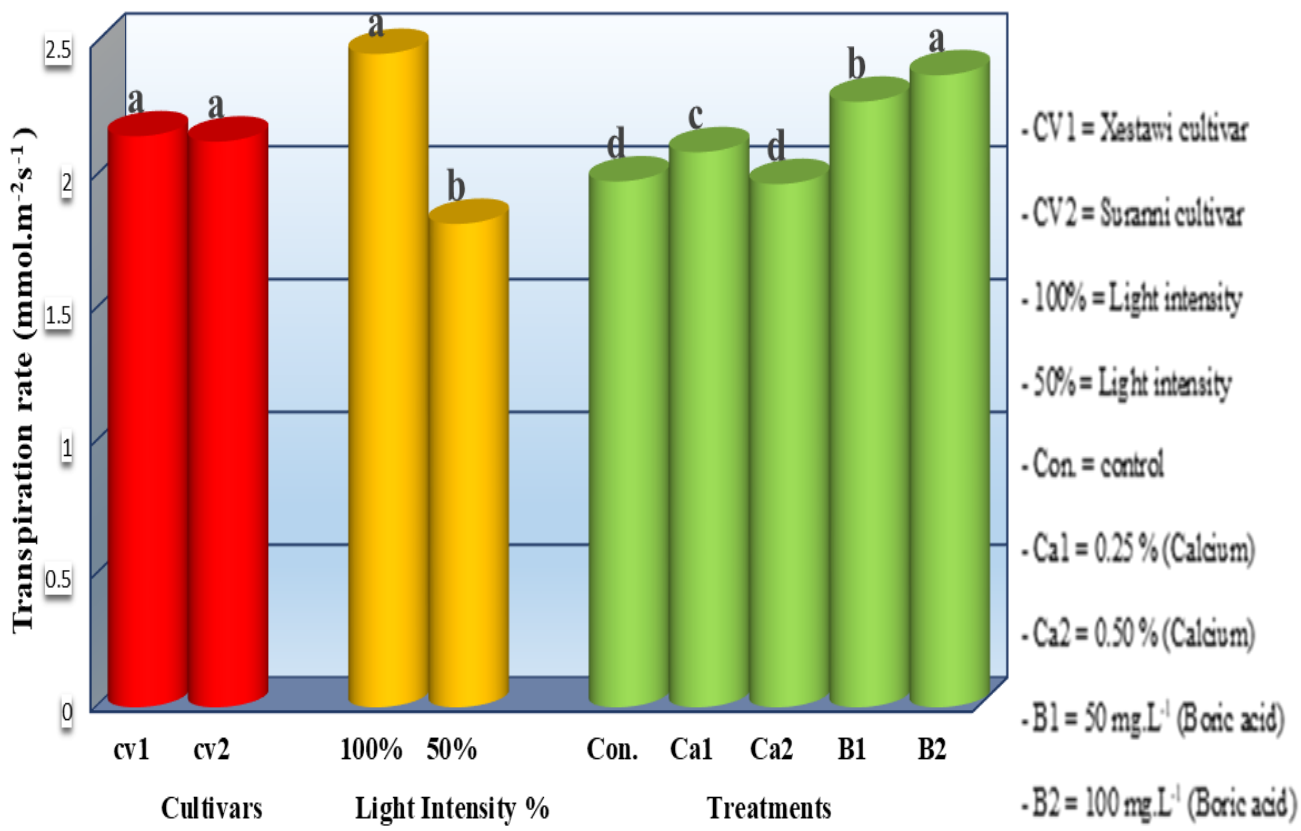
Fig. 1. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on net photosynthesis (μmol.m^{-2}.s^{-1})



Columns with the same letters are not significantly different from each other according to Duncan's multiple range tests at 5% level

Fig. 2. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on transpiration (mmol.m^{-2}.s^{-1}).

Table (1) illustrates that the interaction between cultivars and light intensity indicated that Suranni had the higher value of net photosynthesis (11.70 μmol.m^{-2}.s^{-1}) when exposed to 100% light intensity compared with other interaction. Also the same table show that the interactions between the cultivars and treatments recorded the higher value of net photosynthesis in Suranni treated with 0.50% calcium, which significantly differ from some interactions. Data cleared that the interaction between the light intensity 100% and treated with 100 mg.L^{-1} boric acid had the highest net photosynthesis (12.33 μmol.m^{-2}.s^{-1}) which significantly differs from other treatments except 0.50% calcium when exposed to the same light intensity. Among the interactions of the three studied factors the highest value of net photosynthesis (13.12 μmol.m^{-2}.s^{-1}) was recorded in Suranni, 100% light intensity and 0.50% calcium, and it is significantly higher than the other interactions. While the minimum net photosynthesis (7.78 μmol.m^{-2}.s^{-1}) was observed among the Xestawi, 50% light intensity and 0.50% calcium.

Transpiration rate: Columns in Fig. (2) illustrates that there were no significant differences between the two studied cultivars (Xestawi and Suranni) in transpiration rate. Transpiration rate of olive transplantswere significantly superior when put under 100 % light intensity compared with 50% light intensity. The transpiration rate was also significantly affected by boric acid levels. The transplants sprayed with 100 mg.L^{-1} boric acid showed higher transpiration rate as compared with the other treatments. While the lower transpiration rate was recorded from untreated transplants and those treated with 0.50% calcium.

It is obvious in the interaction between the cultivars and light intensity in table (2), the transpiration rate recorded higher value (2.57 mmol.$m^{-2}s^{-1}$) in Xestawi when exposed to 100% light intensity as compared with other interaction treatments. Whereas in the same table but in the other interactions between cultivars and treatments, higher value of transpiration rate (2.57 mmol.m^{-2}.s^{-1}) was seen in Xestawi, 100 mg.L^{-1} boric acid, which was significantly different from the other treatments. But the lower rate of transpiration appeared in interaction of Xestawi and 0.50% calcium. Data showed that the interaction between the light intensity 100% and treatments with 50 mg.L^{-1} boric acid had a significant and the highest transpiration rate (2.71 mmol.m^{-2}.s^{-1}).The transpiration rate due to the interaction among the three factors (cultivars, light intensity and treatments),was significant among all treatments. The maximum value was (2.99 mmol.m^{-2}.s^{-1}) shown in Xestawi, 100% light intensity and 50 mg.L^{-1} boric acid. While the minimum value (1.44 mmol.$m^{-2}s^{-1}$) of transpiration rate was found in Xestawi, 50% light intensity and 0.25% calcium.

Stomata conductance rate: There were no significant differences between Xestawi and Suranni cultivar on stomata conductance rate (Fig. 3). However, in the same figure, stomata conductance rate of olive transplants under 100% light intensity significantly surpassed transplants under 50% light intensity. Stomata conductance rate of olive transplants treated with 100 mg.L^{-1} boric acid increased significantly and it was different from all other treatments.

The interaction between cultivars and light intensity showed that Xestawi and 100% light intensity significantly influenced stomata conductance rate (103.08 μmol.m^{-2}.s^{-1}) which was the higher value of stomata conductance rate (Table 3). There were also significant differences among all interactions treatments. Results of Xestawi cultivar foliar spray with 100 mg.L^{-1} boric acid increased stomata conductance rate (105.96 μmol.m^{-2}.s^{-1}) when compared with the other treatments in the interaction between cultivars and treatments. Concerning the interaction between light intensity and treatments stomata conductance rate, showed the maximum stomata conductance rate (110.25 μmol.m^{-2}.s^{-1}) when transplants were exposed to 100% light intensity and 50 mg.L^{-1} boric acid. This value was significantly different from all other interaction treatments. According to the results, the highest value (123.66 μmol.m^{-2}.s^{-1}), among the interaction of the three studied factors, could be seen in Xestawi, 100% light intensity and 50 mg.L^{-1} boric acid which was significantly superior to stomata conductance rate of all other interactions. On the other hand, the lowest stomata conductance rate (60.50 μmol.m^{-2}.s^{-1}) was recorded by the interactions among Xestawi cultivars, 50% light intensity and 0.25% calcium.

Table 1. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on net photosynthesis (μmol.m^{-2}.s^{-1}).

Cultivars	Light intensity	Treatments					Cultivars × Light intensity	Effect of cultivars
		Control	Calcium %		Boric acid mg.L^{-1}			
			0.25	0.50	50	100		
Xestawi	100 %	10.72 d-f	10.06 f-h	11.17 cd	10.72 d-f	12.28 b	10.99 b	9.64 b
	50 %	8.81 j	7.80 k	7.78 k	8.89 ij	8.17 k	8.29 d	
Suranni	100 %	10.90 de	10.31 e-g	13.12 a	11.79 bc	12.38 b	11.70 a	10.88 a
	50 %	9.72 gh	10.10 f-h	10.26 e-g	9.50 hi	10.76 d-f	10.06 c	
Cultivars × Treatments	Xestawi	9.76 cd	8.93 e	9.47 de	9.80 cd	10.22 bc	Effect of light Intensity	
	Suranni	10.31 b	10.20 bc	11.69 a	10.64 b	11.57 a		
Light Intensity× Treatments	100 %	10.81 b	10.18 c	12.14 a	11.25 b	12.33 a	11.34 a	
	50 %	9.26 d	8.95 d	9.02 d	9.19 d	9.46 d	9.18 b	
Effect of treatments		10.03 b	9.56 c	10.58 a	10.22 b	10.89 a		

Means of each factor and their interactions followed by the same letter-s are not significantly different from each other, according to Duncan's multiple ranges test at 5% level

Table 2. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on transpiration (mmol.m^{-2}.s^{-1}).

Cultivars	Light intensity	Treatments					Cultivars × Light intensity	Effect of cultivars
		Control	Calcium %		Boric acid mg.L^{-1}			
			0.25	0.50	50	100		
Xestawi	100 %	2.38 e	2.53 cd	2.14 f	2.99 a	2.82 b	2.57 a	2.15 a
	50 %	1.64 jk	1.44 l	1.52 kl	1.75 ij	2.33 e	1.73 d	
Suranni	100 %	2.08 fg	2.59 c	2.40 de	2.43 de	2.32 e	2.36 b	2.13 a
	50 %	1.85 hi	1.82 hi	1.83 hi	1.97 gh	2.05 fg	1.90 c	
Cultivars × Treatments	Xestawi	2.01 d	1.98 d	1.83 e	2.37 b	2.57 a	Effect of light intensity	
	Suranni	1.96 d	2.20 c	2.11 c	2.20 c	2.18 c		
Light intensity × Treatments	100 %	2.23 c	2.56 b	2.27 c	2.71 a	2.57 b	2.46 a	
	50 %	1.74 e	1.63 f	1.67 ef	1.86 d	2.19 c	1.82 b	
Effect of treatments		1.98 d	2.09 c	1.97 d	2.28 b	2.38 a		

Means of each factor and their interactions followed by the same letter-s are not significantly different from each other, according to Duncan's multiple ranges test at 5% level.

Table 3. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on stomata conductance (µmol.m^{-2}.s^{-1})

Cultivars	Light intensity	Treatments					Cultivars × Light intensity	Effect of cultivars
		Control	Calcium %		Boric acid mg.L^{-1}			
			0.25	0.50	50	100		
Xestawi	100 %	94.07 d	100.91 c	84.50 e	123.66 a	112.27 b	103.08 a	88.19 a
	50 %	68.98 h	60.50 i	63.60 i	73.82 g	99.65 c	73.31 d	
Suranni	100 %	82.67 ef	101.27 c	100.92 c	96.84 cd	93.52 d	95.04 b	88.03 a
	50 %	78.23 fg	77.84 fg	77.86 fg	84.03 e	87.21 e	81.03 c	
Cultivars × Treatments	Xestawi	81.52 d	80.70 d	74.05 e	98.74 b	105.96 a	Effect of light intensity	
	Suranni	80.45 d	89.55 c	89.39 c	90.43 c	90.36 c		
Light intensity × Treatments	100 %	88.37 d	101.09 b	92.71 c	110.25 a	102.89 b	99.06 a	
	50 %	73.60 f	69.17 g	70.73 fg	78.92 e	93.43 c	77.17 b	
Effect of treatments		80.98 d	85.13 c	81.72 d	94.58 b	98.16 a		

Means of each factor and their interactions followed by the same letter-s are not significantly different from each other, according to Duncan's multiple ranges test at 5% level

Table 4. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on water use efficiency (µmol. mol^{-1}).

Cultivars	Light intensity	Treatments					Cultivars × Light intensity	Effect of cultivars
		Control	Calcium %		Boric acid mg.L^{-1}			
			0.25	0.50	50	100		
Xestawi	100 %	4.53 g	3.98 h	5.23 c-e	3.58 i	4.36 g	4.33 c	4.61 b
	50 %	5.37 a-d	5.42 a-c	5.11 de	5.08 e	3.50 i	4.89 b	
Suranni	100 %	5.26 b-e	3.98 h	5.46 a-c	4.84 f	5.34 b-e	4.97 b	5.23 a
	50 %	5.26 b-e	5.52 ab	5.60 a	5.82 f	5.22 c-e	5.48 a	
Cultivars × Treatments	Xestawi	4.95 c	4.70 d	5.17 b	4.33 e	3.93 f	Effect of light intensity	
	Suranni	5.26 b	4.75 d	5.53 a	5.33 cd	5.28 b		
Light intensity × Treatments	100 %	4.89 b	3.98 d	5.34 a	4.21 c	4.85 b	4.65 b	
	50 %	5.31 a	5.47 a	5.35 a	5.45 b	4.36 c	5.19 a	
Effect of treatments		5.10 b	4.72 c	5.35 a	4.83 d	4.60 d		

Means of each factor and their interactions followed by the same letter-s are not significantly different from each other, according to Duncan's multiple ranges test at 5% level

The interaction between cultivars and light intensity in (Table 4) revealed that the Suranni cultivar under 50% light intensity gave the higher value of water use efficiency (5.48 μmol.mol^{-1}) when compared with the other treatments. Also the same table shows that the interaction between the cultivars and treatments of water use efficiency manifests significant differences. However, a high value (5.53 μmol.mol^{-1}) of water use efficiency was noticed in Suranni treated with 0.50% calcium. The higher value (5.47 μmol.mol^{-1}) of water use efficiency was recorded in transplants that were exposed to 50% light intensity and 0.25% calcium. While the lowest value (3.98 μmol.mol^{-1}) was recorded from the interaction between 100% light intensity and 0.25% calcium. The interaction among Suranni, 50% light intensity and 0.50% calcium, was significant by recording higher value (5.60 μmol.mol^{-1}). But the lowest value (3.50 μmol.mol^{-1}) of water use efficiency was recorded in most interaction of Xestawi, 50% light intensity and 100 mg.L^{-1} boric acid.

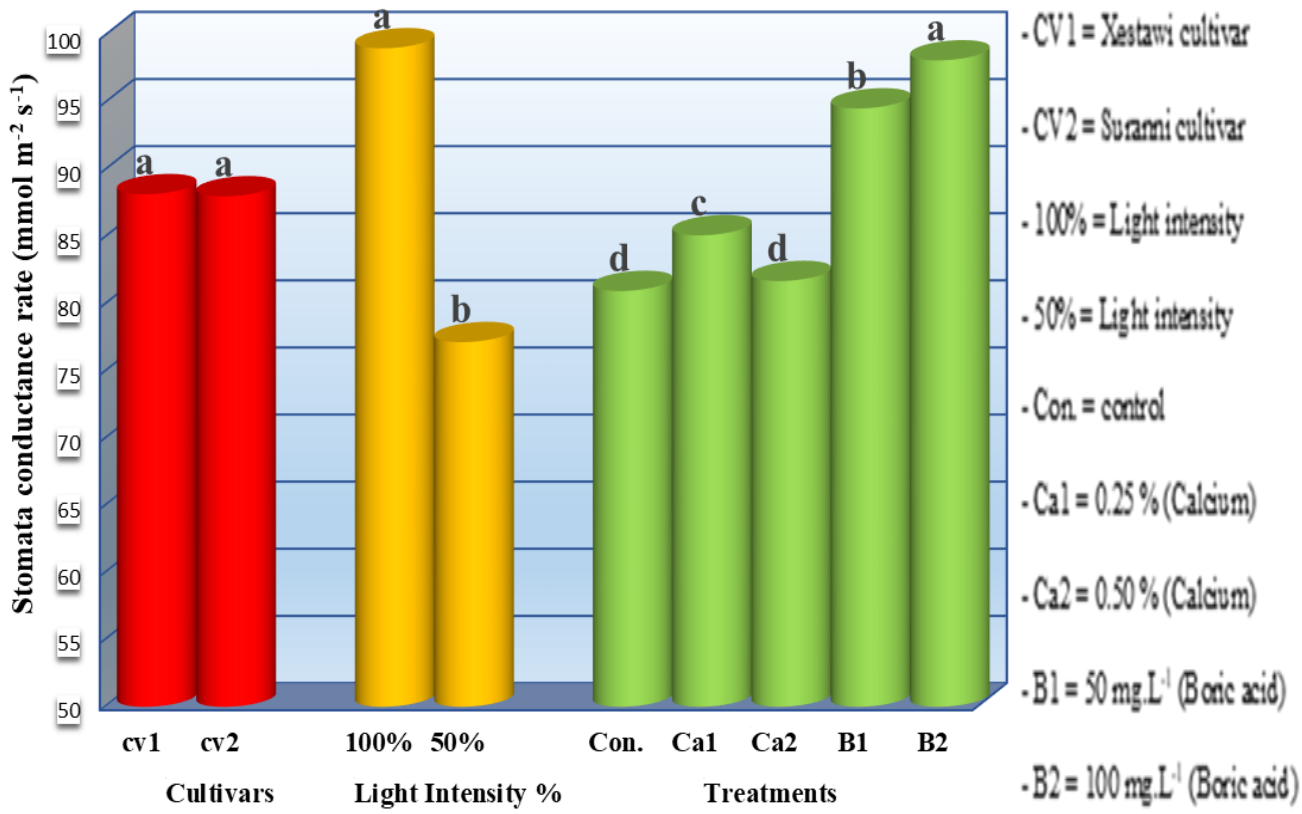
Water use efficiency: Suranni significantly had the highest water use efficiency as compared with Xestawi (Fig. 4). In other words, when olive transplants were exposed to two levels of light intensity, water use efficiency was significantly higher in transplants exposed to 50% light intensity than those exposed to 100% light intensity.

It is also obvious from the data that the higher significant value of water use efficiency was recorded in transplants sprayed with 0.50% calcium, while the minimum water use efficiency was recorded in 100 mg.L^{-1} boric acid.

Stomata density: It was found that there was no significant difference between the both cultivars Xestawi and Suranni on stomata density (Table 5). Concerning specific effect of light intensity, it was quite clear that the highest significant number of stomata was founded in 100% light intensity. While the higher number of stomata were observed when the transplants were treated with 0.25% calcium. But the differences between treatments were not significant, and the lower number of stomata appeared from untreated transplants. The interaction between cultivars and light intensity exhibited that the Xestawi and Suranni exposed to 100% light intensity showed significant differences from 50% light intensity by recording (418.00) and (404.00) number of stomata respectively. Xestawi cultivar treated as foliar spray with 0.25% calcium had significantly positive effect on stomata density in the interactions between the cultivars and treatments. On the other hand lower number of stomata recorded with untreated Xestawi cultivar transplants. Also the interactions between the light intensity and treatments (100% light intensity with 0.25% calcium) gave significantly the highest number of stomata (438.00). While the less number of stomata was found from light intensity 50% and untreated transplants. Concerning the interactions among the factors cultivar Xestawi, 100% light intensity and 0.25% calcium gained the highest number of stomata (454.00) which was significant in comparison with the lowest number of stomata found in Xestawi, 50% light intensity and control (339.00) and most other interactions among the three factors.

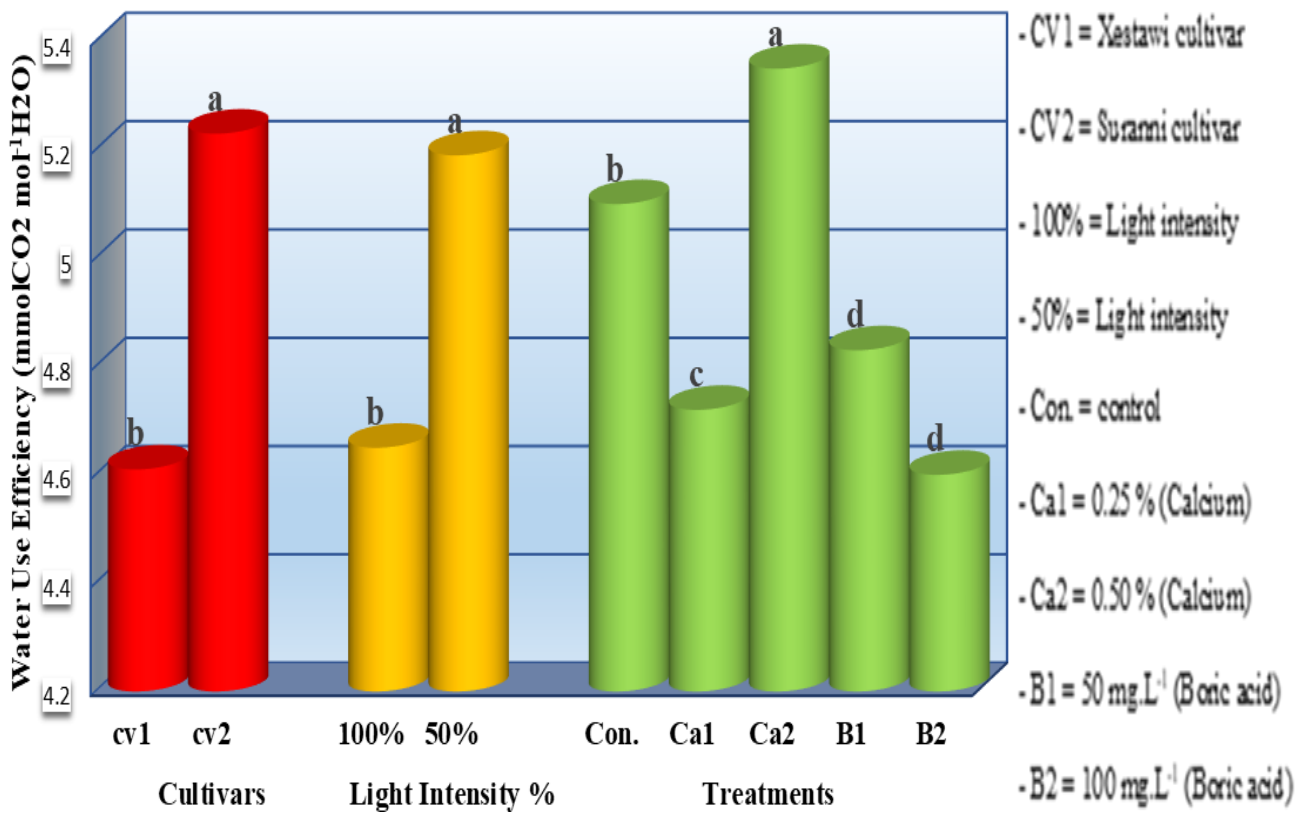
Guard cell thickness: Results in table (6) shows that Xestawi cultivar had significantly superior in guard cell thickness above Suranni cultivar. Also when the transplants exposed to 100% light intensity had significantly higher value of guard cell thickness. Data cleared that transplants treated foliar sprayed with 0.50% calcium and untreated transplants recorded significantly the highest guard cell thickness, respectively. It was explained that the guard cell thickness was significantly increased in Xestawi exposed to 100% light intensity (8.10 μm) in comparison with other interactions. On the other hand, the guard cell thickness in Xestawi transplants sprayed with 0.50% calcium had significantly higher (8.21 μm) guard cell thickness **which was different** from most of interactions. Also the interactions between the light intensity and treatments showed that the higher value of guard cell thickness (7.94 μm) was recorded in transplants exposed to 100% light intensity and treated spray 0.50% calcium. The less value (6.62 μm) of guard cell thickness was recorded at interaction between 50% light intensity and 0.25% calcium or 50 mg.L^{-1} boric acid. As for the interactions among the cultivars, light intensity and treatments, the same table explained that the Xestawi with 100% light intensity and 100 mg.L^{-1} boric acid significantly affected on guard cell thickness and listed the highest value (8.36 μm) while the lowest value (5.74 μm) was recorded in Suranni, 50% light intensity and 50 mg.L^{-1} boric acid.

Chlorophyll *a*: Suranni was significantly influenced on chlorophyll *a* (Table 7). When it was exposed to different light intensity, the higher significant value of chlorophyll *a* was in transplants exposed to 100% light intensity. On the other hand when the transplants treated or untreated with different treatments (calcium and boric acid) in the same table cleared that there were no significant differences between the treatments. The higher chlorophyll *a* content was in transplants treated with 0.50% calcium. Results presented realized that the interactions between the cultivars and light intensity significantly affected chlorophyll *a*, It gave (30.58 μg.g^{-1}) as a higher value of chlorophyll *a* from the interaction between Suranni and 100% light intensity in comparison with the other treatments. The interactions between the cultivars and the treatments showed that the Suranni transplants sprayed with 0.50% calcium had significantly higher (30.90 μg.g^{-1}) chlorophyll *a* which was differed from most of interactions. Transplants exposed to 100% light intensity and treated foliar spray with 0.25% calcium gave significantly maximum chlorophyll *a*. But the lower chlorophyll *a* was recorded in the interaction between 50% light intensity and 0.25% calcium. The interactions among the three factors for chlorophyll *a* showed that the highest value appeared in Suranni, 100% light intensity and control which was (35.05μg.g^{-1}). While the minimum chlorophyll *a* value was (10.75μg.g^{-1}) among Xestawi, 50% light intensity and control.



Columns with the same letters are not significantly different from each other according to Duncan's multiple range tests at 5% level

Fig. (3). Effect of light intensity, spraying of calcium and boric acid elements and their interactions on stomata conductance (μmol.m^{-2}.s^{-1}).



Columns with the same letters are not significantly different from each other according to Duncan's multiple range tests at 5% level.

Fig. 4. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on water use efficiency (WUE) (μmol.mol^{-1}).

Table 5. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on stomata density.

Cultivars	Light intensity	Treatments					Cultivars × Light intensity	Effect of cultivars
		Control	Calcium %		Boric acid mg.L^{-1}			
			0.25	0.50	50	100		
Xestawi	100 %	388.00 c-e	454.00 a	398.00 b-e	415.00 a-c	437.00 ab	418.00 a	397.00 a
	50 %	339.00 f	405.00 b-d	366.00 d-f	365.00 d-f	403.00 b-d	376.00 b	
Suranni	100 %	405.00 b-d	421.00 a-c	401.00 b-d	408.00 b-d	384.00 c-f	404.00 a	385.00 a
	50 %	357.00 c-f	354.00 ef	378.00 c-f	380.00 c-f	362.00 d-f	366.00 b	
Cultivars × Treatments	Xestawi	364.00 c	430.00 a	382.00 c	390.00 bc	420.00 ab	Effect of light intensity	
	Suranni	381.00 bc	388.00 c	390.00 bc	394.00 bc	373.00 c		
Light intensity × Treatments	100 %	397.00 b-d	438.00 a	400.00 b-d	412.00 ab	411.00 a-c	411.00 a	
	50 %	348.00 e	380.00 de	372.00 de	373.00 de	383.00 c-e	371.00 b	
Effect of treatments		372.00 b	409.00 a	386.00 b	392.00 ab	397.00 ab		

Means of each factor and their interactions followed by the same letter-s are not significantly different from each other, according to Duncan's multiple ranges test at 5% level

Table 6. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on guard cell thickness (µm).

Cultivars	Light intensity	Treatments					Cultivars × Light intensity	Effect of cultivars
		Control	Calcium %		Boric acid mg.L^{-1}			
			0.25	0.50	50	100		
Xestawi	100 %	8.13 a-c	7.99 a-d	8.22 ab	7.68 b-e	8.36 a	8.10 a	7.91 a
	50 %	8.25 ab	7.21 e-g	8.21 ab	7.51 c-f	7.43 d-g	7.72 b	
Suranni	100 %	7.41 d-g	7.16 e-g	7.67 b-e	6.92 fg	6.93 fg	7.22 b	6.75 b
	50 %	6.89 fg	6.03 h	6.82 g	5.74 h	5.94 h	6.28 c	
Cultivars × Treatments	Xestawi	8.19 a	7.60 bc	8.21 a	7.59 bc	7.89 ab	Effect of light intensity	
	Suranni	7.15 d	6.59 e	7.24 cd	6.33 e	6.43 e		
Light intensity × Treatments	100 %	7.77 a	7.58 ab	7.94 a	7.30 b	7.18 b	7.55 a	
	50 %	7.57 ab	6.62 c	7.51 ab	6.62 c	7.15 b	7.09 b	
Effect of treatments		7.67 a	7.09 b	7.73 a	6.96 b	7.17 b		

Means of each factor and their interactions followed by the same letter-s are not significantly different from each other, according to Duncan's multiple ranges test at 5% level.

Table 7. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on chlorophyll a (µg.g^{-1}).

Cultivars	Light intensity	Treatments					Cultivars × Light intensity	Effect of cultivars
		Control	Calcium %		Boric acid mg.L^{-1}			
			0.25	0.50	50	100		
Xestawi	100 %	11.65 hi	17.38 f-h	12.79 hi	17.14 f-i	20.30 e-g	15.85 c	14.61 b
	50 %	10.75 i	14.05 g-i	12.46 hi	15.06 g-i	14.55 g-i	13.37 c	
Suranni	100 %	35.05 a	32.03 ab	31.88 ab	28.64 b-d	25.31 c-e	30.58 a	27.69 a
	50 %	25.96 b-e	22.11 ef	29.91 a-c	22.44 ef	23.54 de	24.79 b	
Cultivars × Treatments	Xestawi	11.20 e	15.72 c	12.63 de	16.10 c	17.42 c	Effect of light intensity	
	Suranni	30.50 a	27.07 ab	30.90 a	25.54 b	24.43 b		
Light intensity × Treatments	100 %	23.35 ab	24.70 a	22.34 a-d	21.85 a-d	22.81 a-c	23.01 a	
	50 %	18.36 cd	18.08 d	21.19 a-d	19.79 b-d	19.04 b-d	19.29 b	
Effect of treatments		20.85 a	21.39 a	21.76 a	20.82 a	20.92 a		

Means of each factor and their interactions followed by the same letter-s are not significantly different from each other, according to Duncan's multiple ranges test at 5% level

Table 8. Effect of light intensity, spraying of calcium and boric acid elements and their interactions on chlorophyll b (µg.g^{-1}).

Cultivars	Light intensity	Treatments					Cultivars × Light intensity	Effect of cultivars
		Control	Calcium %		Boric acid mg.L^{-1}			
			0.25	0.50	50	100		
Xestawi	100 %	2.48 d-h	3.21 bc	2.33 e-i	2.75 c-g	3.28 a-c	2.81 b	2.49 b
	50 %	1.73 i	2.26 g-i	2.01 hi	2.54 d-h	2.32 f-i	2.17 c	
Suranni	100 %	3.04 b-d	3.86 a	2.91 b-f	3.40 ab	3.46 ab	3.33 a	3.22 a
	50 %	2.96 b-e	3.17 bc	2.87 b-g	3.30 a-c	3.25 a-c	3.11 ab	
Cultivars × Treatments	Xestawi	2.11 d	2.74 c	2.17 d	2.64 c	2.80 c	Effect of light intensity	
	Suranni	2.99 bc	3.52 a	2.89 c	3.35 ab	3.36 ab		
Light intensity × Treatments	100 %	2.72 c-e	3.54 a	2.60 de	3.07 bc	3.37 ab	3.06 a	
	50 %	2.39 e	2.72 c-e	2.46 e	2.92 cd	2.79 c-e	2.66 b	
Effect of treatments		2.55 b	3.13 a	2.53 b	2.99 a	3.08 a		

Means of each factor and their interactions followed by the same letter-s are not significantly different from each other, according to Duncan's multiple ranges test at 5% level

Chlorophyll *b*: It was obvious from Table 8 that Suranni was significantly higher than Xestawi in chlorophyll *b*. Transplants when exposed to 100% light intensity significantly surpassed from transplants under 50% light intensity in chlorophyll *b*. Treated transplants with 0.25% calcium had significantly higher chlorophyll *b*. It is worth noting that there were no significant differences between the treatments except the transplants treated spray with 0.50% calcium. Also results indicated that Suranni and 100% light intensity gave (3.33 µg.g^{-1}) which was the higher value of chlorophyll *b*. However, lower chlorophyll *b* was in the interaction between Xestawi cultivar that exposed to 50% light intensity. Results of Suranni cultivar transplants which was treated foliar spray with 0.25% calcium increased chlorophyll *b* value (3.52 µg.g^{-1}) when compared with lower value (2.11 µg.g^{-1}) in Xestawi cultivar and control. Concerning the interaction between light intensity and treatments appeared when transplants wee exposed to 100% light intensity and 0.25% calcium, had the maximum chlorophyll *b* value (3.54 µg.g^{-1}) which was significantly different from other treatments except chlorophyll *b* under 100% light intensity and 100 mg.L^{-1} boric acid treatment. The highest value (3.86 µg.g^{-1}) of chlorophyll *b* showed among the interaction of Suranni, 100% light intensity and 0.25% calcium which was significantly superior from most other interactions among the all features under study. The minimum chlorophyll *b* was recorded from the interaction among Xestawi cultivar, 50% light intensity and untreated transplants.

Discussion

The present investigation was to study the influence of light intensity and some chemical composition on some physiological features of olive transplants (*Olea europaea* L.) cultivars Xestawi and Suranni. The results effectively confirmed that the light intensity (100%) had significant effect on all physiological parameters including the net photosynthesis, transpiration, stomata conductance, stomata density, guard cell thickness, chlorophyll *a* and chlorophyll *b*, as shown in Tables (1, 2, 3, 5, 6, 7, 8) respectively when compared with (50%) light intensity. The latter gave only higher water use efficiency as shown in Table (4). Leaves are the essential organ of photosynthesis and transpiration. The level of stomata, mesophyll cells, and chloroplasts development directly influence net photosynthesis and transpiration rate growth (Li, 2006). The increase in net photosynthesis and transpiration stomata conductance directly depends on stomatal density. Olive leaves have a hypostomatic condition which are mainly on the lower surface. The stomatal density varies amongst different olive cultivars (Bongi *et al.*, 1987). Hence, the increase in the rate of photosynthesis and transpiration rate proved in this study may be due to the positive effect of transplants being exposed to 100% light intensity on stomata conductance, stomata density and chlorophyll *a* and chlorophyll *b* (Tables 1, 2, 3, 5, 7, 8) respectively. Also the increase on net photosynthesis and transpiration under 100% light intensity might be due to the morphological changes in leaves and olive tree adapts well to high light intensity compared with other fruit trees by increasing the number of stomata, more cuticle thickness and more palisade parenchyma layers (Gregoriou *et al.*, 2007). Similar results have been recorded for olive by (Higgins *et al.*, 1992; Bongi & Palliotti, 1994). On the other hand, the thicker leaves under high light intensity, have higher level of surface area in which the chloroplast facing the intercellular space leads to an increase the intercellular space. The byproduct is an increase in CO_2 diffusion area. This leads to an increase of CO_2 assimilation (Lestari & Setiawati, 2018).

It is clear that the palisade tissue has better ability to light penetration of chloroplasts than spongy tissue because the former enhances the light capture by scattering light. Thus, it is supposed that leaves growing under high light intensity have a thicker palisade parenchyma creating an efficient structure in terms of photosynthesis. Moreover, high light leaves have high stomatal density which progress their CO_2 uptake (Evans, 1999). On the other hand, it was observed from the present study that spraying the transplants with calcium and boric acid leads to a significant increase in most photosynthetic features. The higher net photosynthesis,

transpiration, stomata conductance were also observed in transplants treated with 100 mg.L^{-1} boric acid as shown in Tables (1, 2 and 3) respectively. Boron is a major element for all vascular plants in which deficiency or toxicity causes impairments in several metabolic and physiological processes (Camacho-Cristobal *et al*., 2008). The physiological effects of boron toxicity include lower leaf chlorophyll contents and reduced net photosynthesis (Reid, 2007). Boron toxicity is more difficult to manage than its deficiency (Takano *et al*., 2008). Boron deficiency cause an accumulation of carbohydrate in leaves. Assimilation of CO_2 may be regulated by the immoderate accumulation of starch and hexoses via direct interference with chloroplast function and indirect repression of photosynthetic enzymes (Han *et al*., 2008). Boron deficiency leads to the decrease of stomatal conductance and water potential in turnip (Hajiboland &Faranghi, 2011). Water use efficiency reduces under boron deficiency due to a decline in stomatal conductance. Frequently, when stomatal closure is stimulated by boron deficiency, there is a relative stability in leaf Water use efficiency as the reduction in transpiration is slightly greater than a reduction in net photosynthesis. Any potential benefits of reduced stomatal conductance on water use efficiency seem to be negated by a reduction in net carbon fixation (Tavallali, 2017).

El-Shintinawy, (1999) proved that low supply of boron in sunflower plant decreased net photosynthesis rate. Also boron deficiency decreased net photosynthesis in cotton plants (Zhao & Oosterhuis, 2002). It has been confirmed that boron deficiency causes a decrease in leaf stomata conductance and net photosynthesis. The low net photosynthesis is related to the decrease in stomata conductance and that leads to decrease in transpiration. Boron deficiency also leads to decrease in chlorophyll content by accumulation of starch in leaf. Probably, starch deactivates chloroplast structure. In addition, application of boron brings about an increase in the net photosynthesis rate. The above increasing rate has more relationship with high stomata conductance in the presence of an enough supply of boron (Han *et al*., 2008). High stomata conductance also increases transpiration rate. This shows that the application of boron in plant affects the morphology of stomata (length and width). Stomata become longer and wider due to increasing of boron in cell wall especially the guard cells (Shaaban, 2010). Although increasing net photosynthesis rate leads to an increase of leaf chlorophyll content, this increase stimulates the stomata to open wider. Therefore, the gasses will be better circulated under increasing the application of boron (Pinho *et al*., 2010). Boron deficiency advocates an alteration in the photosynthetic enzymes that are probably involved in a decrease in the net photosynthesis indirectly (Sharma & Ramchandra, 1990). So, the reason for the increase of the above mentioned physiological processes when transplants were sprayed with 100 mg.L^{-1} boric acid was attributed to the active role in the pathway of these vital processes and their stimulated enzymes. While higher rates of water use efficiency, stomata density, guard cell thickness and chlorophyll *b* were found in transplants treated with calcium concentrations (0.25% and 0.50%) as shown in Tables (4, 5, 6 and 8) respectively. These results may be attributed to the role of calcium related to regulatory mechanisms that help plants to adapt to unfavorable environmental conditions and play an important role in maintaining the stability of cell membrane phospholipids and proteins (Upadhyaya *et al*., 2011). Similar results were observed by Amor *et al*., (2010) & Xu *et al*., (2013) that chlorophyll content was enhanced by calcium chloride application. While the net photosynthesis and stomata conductance with calcium chloride pretreatment were higher than the control. They attributed the reason to the fact that calcium could relieve stress-induced damages and increase photosynthetic performance (Li *et al*., 2017). It might also be due to fact that calcium treatment prevents the dehydration damage of cellular structure by maintaining the osmotic strength of the cytoplasm in plants (Yang *et al*., 2016). Also calcium may regulate stomata movement and decrease respiration intensity (Jones& Lunt, 1967).

Conclusion

It is concluded that the light intensity, calcium in both concentration and boric acid (100 mg.L^{-1}) influence some leaf physiological processes, stomata density and chlorophyll content of olive transplants. Light intensity 100% resulted in higher net photosynthesis, transpiration rate, stomata conductance, stomata density, guard cell thickness and chlorophyll content (*a* and *b*) compared to other light intensity. While 50% light intensity lead to an increase in water use efficiency. The results also showed that boric acid foliar at (100 mg.L^{-1}) had higher net photosynthesis, transpiration rate and stomata conductance. While calcium foliar of both concentrations (0.25 and 0.50 %) increased the water use efficiency, stomata density, guard cell thickness and chlorophyll *b*.

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(Received for publication 22 September 2018)