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Effects of Shading and Fertiliser on the Growth and Antioxidant Content of Olives (*Olea europaea* L.)

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ABSTRACT

The olive tree not only provides fruits but also wood and nutrition such as oleuropein. Olive leaves can be made into tea by 'withering' (drying) the fresh leaves. An experiment was conducted to observe the growth and evaluate the antioxidant content of olives using different types of fertilisers. The plants were grown under partial shading and full exposure to sunlight (50% and 0% - no shading); NPK fertiliser or commercial compost was used to enrich the planting media. The results showed that plants that grew in the shade are taller and have better foliage, while use of commercial compost resulted in better growth of branches and leaves. Older leaves contained higher levels of oleuropein and ascorbic acid. Exposure to sunlight increased ascorbic acid content in older leaves compared with that of shaded plants.

Keywords: Fertiliser, shading, growth performance, olive, antioxidant

INTRODUCTION

Olive (*Olea europaea* L.) is a plant that originated from the Mediterranean region and was introduced to tropical regions such as Indonesia. The country with its tropical humid climate is a suitable place to grow olives, but it is believed that the growth as well as productivity of the olive tree would be different. Furthermore, micro-climatic conditions due to the planting system may have some influence. Olives are commonly planted in a monoculture system, but can be grown in a mix-farming system due to scarcity of land. In the monoculture system, the plants are grown in full exposure of sunlight. In the mix-culture system, the

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plants grow under many different trees that might reduce their exposure to sunlight, such as growing under a shade.

Differences in environmental conditions of both systems may affect plant growth, i.e. its height, number of leaves, or number of branches. Environmental condition is also affected by soil fertility. The main nutrients needed by plants are nitrogen (N), phosphorus (P), potassium (K) (López-Granados et al., 2004).

The olive tree relies on secondary metabolites to interact with its environment for adaptation and defence. Phenolic compounds are among the known substances that protect the tree against biotic and abiotic stresses (Battacharya et al., 2010). The most abundant phenolic compounds in olive are phenolic acids, phenol alcohol, flavonoids, and secoiridoids (Servili et al., 2004), and oleuropein is derived from secoiridoid (De La Torre-Carbot et al., 2005; Silva et al., 2006).

Olives have medicinal values and the substance extracted from the fruits and twigs is considered part of a healthy diet due to their secondary metabolite. Apart from its role in plant defence, oleuropein has antioxidant, anti-inflammatory, anti-cancer, antiviral, and antimicrobial properties (Omar, 2010; Durlu-özkaya & Özkaya, 2011). Oleuropein is found not only in the olive fruit, but also in the leaves and seeds (Ryan et al., 2002), with the highest concentration found in fruits (pastes and pulps) and in leaves (Silva et al., 2006). However, the fruits are largely processed for olive oil (Vossen, 2013). Olive leaves are another source of oleuropein, and can be processed into tea leaves, through withering and drying of fresh leaves. This must be supported by increased production of the leaves.

Ascorbic acid is as an antioxidant and a co-factor of certain enzyme involved in photoprotection of plants (Smirnoff, 1996; Khan et al., 2012; Gallie, 2013). Therefore, plant performance can be evaluated through its phenol and ascorbic acid content. It is assumed that oleuropein as well as ascorbic acid levels would vary in olive trees planted under full sunlight and under shady conditions.

There is limited information on the effect of tropical humidity on growth and antioxidant content of olives, especially the effect of shading and fertiliser use. Therefore, this research examined the growth of olive trees in various conditions, i.e. shaded growth in combination with inorganic or organic fertilisers, and the effect of treatments on their oleuropein and ascorbic acid content.

MATERIALS AND METHODS

Experimental site and plant material

The experiment was conducted in Depok, West Java, Indonesia between September 2014 and March 2015. Two year-old olive trees were planted in pots of 45 cm in diameter, one plant per pot. The distance between the pots was 70 x 70 cm.

Experimental design

This research used split plot design to measure the growth of the olive trees as well as oleuropein and ascorbic acid content of the leaves; shading as the main plot and fertiliser regime as the sub plot. In order to analyse oleuropein and ascorbic acid concentration in fresh olive leaves, different types of leaves were placed in the sub-plots. The shading has two levels: 50% shading (N1) and full exposure to sunlight (N2). Two types of fertilisers were used: NPK 20:10:10 (P1) and commercial compost (P2). The soil was fertilised using NPK fertiliser , on day-0 with 105 g/pot and on day-56 with 50 g/pot. Compost (2 kg/pot) was mixed with the soil before being used as media. Young leaves (D1, the first to the sixth leaves from the tip) and old leaves (D2, the seventh to the 12th leaves) were collected.

Growth variable measurement

Growth of the plants was observed every three weeks for four months. Plant height was measured from the base of the stem above the ground to the tip of the shoot. Stem diameter was observed at 3 cm above ground using vernier callipers.

Olive tea leaves processing by withering fresh leaves

Fresh leaves were collected to be made into tea every week beginning from week 2 after treatment (WAT) up to 15 weeks. The method followed the processing procedure for *Camelia sinensis* leaves into green tea (Shi & Schlegel, 2012) with temperature modification. The leaves were dried under sunlight for 1-2 hour(s), then in room temperature for 1-2 day(s). Leave rolling was executed at 40°C for no longer than 15 minutes, followed by further drying in an oven at 40°C for 90 minutes.

Analysis of biochemical parameters of olives

Determination of oleuropein content in olive leaves. The oleuropein level was analysed using High Performance Liquid Chromatograph (HPLC) following the method described by Bouaziz et al. (2008). Tea leaves were crushed into powder and fresh leaves were minced in a blender for 2 minutes and stored in a dry place until the time for extraction. It was replicated two times. Six grams of each sample was used for the extraction process. After the final centrifugation at 10,000 rpm for 10 minutes, the supernatant was stored at 0°C in darkness before chromatographic analysis. The mobile phase used acetonitrile: water (7: 3) as solvent A; phosphoric acid (0.1% in water) as solvent B. The flow rate of eluates was 0.6 mL/min and injection volume was 20 µL for 50 minutes. Temperature was set at 40°C and detected at 280 nm.

Measuring ascorbic acid level in olive leaves. About 0.5 g fresh leaves (young and old leaves) or tea leaves were macerated with 5 mL of metaphosphoric acid 5% in a mortar and filtered using filter paper up to 10 mL . The sample was titrated with 2.6-dichlorophenol indophenols (2.6-DCIP) reagent 0.025% until a pink end point that persists for 15 seconds was obtained (Rao & Sresty, 2000).

Data Analysis

ANOVA was used to analyse data using SPSS version 19.0, followed by Duncan's Multiple Range Test (DMRT) to determine the difference among treatments. P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Shading effects on microclimate

This research was conducted under two shading conditions, 50% shading and non-shading. The environmental parameters (temperature, light intensity, and humidity) were observed in both conditions. Table 1 shows data collected for several days during mid-day between 12pm and 1pm. Shading treatment changed the microclimate around the plants. In general, there was no noticeable difference in the temperature under shading and under full sunlight; the temperature was considered mild. The relative humidities (RHs) at mid-day ranged between 39% and 53%, where the shaded area had slightly higher RH. Shading treatment also reduced light intensity by between 60% and 65%. In the Mediterranean regions, average temperatures during winter time ranged between 2°C and 18°C and during summer between 10°C and 46°C, with RH averages at 40% and 80% (Chiraz 2013; Orlandi et al., 2013). The climate differences between Mediterranean and the Western part of Indonesia may affect metabolic activities in the plants, although olive trees can grow well in other regions due to their adaptive ability to a wide range of temperature; but the generative phase of olives needs chilling temperature to induce the initiation of flower-buds and to break dormancy (Fabbri et al., 2009; Orlandi et al., 2013).

Table 1
Environmental parameters as influenced by shading

Environmental Parameters	50% Shading	Non-Shading
Temperature (°C)	28.8 - 30	30.3 – 34.3
Light intensity (lux)	6,870- 8,600	19,800 – 21,567
Relative Humidity (%)	41.3 – 52.4	38.9 – 45.1

Effects of shading and fertilizer on growth performance

The olive plants grew from the 3rd week and were observed up to 15th week after treatment (WAT). Analysis of variance on data collected at 15th WAT shows no interaction between shading and the types of fertiliser on all growth parameters, but each single factor did have its effects. Fifty percent shading significantly increased the height of the plant and the number of leaves compared with those under full sunlight. There were more branches and leaves in plants in the media with compost with NPK fertiliser (Table 2). The olive plants that are partially shaded suffered etiolation that increased their height and amount of leaves. The colour of the leaves is also different as well as the branch growth. Olive plants

Table 2
Effects of shading and types of fertiliser on the growth of olive plants

Treatments	Plant Height (cm)	Number of Leaves	Number of Branches
Shading (N)			
50% shading (N1)	147.83[a]	2553.90[a]	59.80[a]
Non-shading (N2)	130.83[b]	2300.50[b]	55.80[a]
Fertiliser (P)			
NPK fertilizer (P1)	133.00[a]	2240.50[b]	54.20[b]
Compost (P2)	145.66[a]	2613.90[a]	61.40[a]

Numbers followed by the same letter in the same column are not significantly different with DMRT test, α=5%. Data were collected at the 15th week after treatment

that are grow in the shade have longer and more flexible branches and the leaves have a lighter green.

When the plants are treated with compost, growth of foliage and branches is better than with NPK. Compost is an organic matter derived from livestock, crop residues, or waste products. These materials will be decomposed and become a source of nutrient for plants (Brinton, 2000). Compost has more nutrients compared with synthetic fertiliser but it needs a longer time to be absorbed by plants due to its decomposition process (Wei & Liu, 2005). Compost influences plant growth directly and indirectly. It indirectly improves the soil condition physically, chemically and biologically. Compost directly provides nutrients by increasing chlorophyll content, improving photosynthesis and hormonal growth responses (Hafez et al., 2015). The NPK fertiliser (20:10:10) as much as 105 g/plant was expected to improve the growth of plants, particularly the foliage. Nitrogen is needed to form chlorophyll and in photosynthetic activity while phosphate is needed for energy storage through the transfer to ATP and ADP, while potassium enhances process of water uptake which has a consequence on cell enlargement (Freihat & Masadeh, 2006). But in this case, the amount and ratio of NPK fertiliser used was not able to increase foliage compared with using compost.

Oleuropein content

Table 3 shows oleuropein concentrations in tea leaves. Statistically, there is no link

Table 3
Oleuropein concentration in olive leaves tea from the plants grown under different growing conditions

Oleuropein concentration in olive leaves tea (mg g^{-1})*			
Shading (N)		Fertilizer (P)	
50% shading (N1)	29.17[a]	NPK fertilizer (P1)	33.93[a]
Non-shading (N2)	34.43[a]	Compost (P2)	29.68[a]

Numbers followed by the same letter in the same column are not significantly different with DMRT test, α=5%. *Means of two replications between shading and fertiliser on oleuropein content. It is indicated that partial shading or the types of fertiliser used do not influence the oleuropein content in tea leaves.

This study proved that there is a high content of oleuropein (in average 31.8 mg g^{-1}) in processed leaves compared with those previously reported. Jemai et al. (2009) reported that oleuropein concentration in dried olive leaves from Tunisia processed at 40°C was 24.4 mg g^{-1}, while Afaneh et al. (2015) obtained 10.0 mg g^{-1} when the leaves from Palestine was processed at 25°C and 1.7 mg g^{-1} at 50°C. It suggested that in general, the climatic conditions in the research site may not be favourable for the normal growth of olive plants, and that they produced phenolic compounds, in this case oleuropein, more abundantly. The concentration of a particular phenolic compound within a plant tissue is dependent on the season and may also vary at different stages of its growth and development (Lynn & Chang, 1990; Ozyigit et al., 2007), and it is influenced by internal as well as external stress. According to Vuong et al. (2013), phenolic compounds are easily degraded by high temperature and light, and are also easily oxidised; that may describe the lower content of oleuropein in leaves originating from the above mentioned Mediterranean countries. Additionally, the three successive drying-phases applied in this research may be more effective in ensuring high oleuropein in the leaves.

In fresh leaves, the oleuropein content was not influenced by the treatments applied The difference is only between young and older leaves (Table 4). Older leaves contain more oleuropein than younger ones. In normal conditions, the concentration of antioxidant and other secondary metabolites in older plant tissues/organs is higher than in younger ones, while under stress conditions the concentration will be even manifold (Abdallah et al., 2013; Majer & Hideg, 2012). This is due to the function of secondary metabolites as self-defensive agent against predators or pathogens, and because the metabolites are synthesised when the cell growth rate starts to decline after the maturity phase (Mazid et al., 2011).

Table 4
Oleuropein concentration in fresh olive leaves

Type of leaves (D)	Oleuropein concentration in fresh olive leaves (mg g^{-1})*
Young leaves (D1)	10.17^{b}
Older leaves (D2)	12.91^{a}

Numbers followed by the same letter in the same column are not significantly different with DMRT test, α=5%. *Means of two replications

Ascorbic acid content

Shading and leaf type influence ascorbic acid levels. Table 5 shows that the level of ascorbic acid in N2D2 (non-shaded older leaves) was significantly higher than in young leaves of both shaded and not shaded plants (N1D1 and N2D1). Old leaves from plants exposed to full sunlight (N2D2) and those under shade (N1D2) have comparable levels of ascorbic acid, although the fomer tends to be more abundant.

When growth rate starts to decline in older leaves, the plant will accumulate more

Table 5
Effects of shading and type of leaves on ascorbic acid concentration in fresh olive leaves

Treatments	Ascorbic acid concentration (mg/g)*
N1D1 (young leaves in 50% shading)	0.16[b]
N1D2 (older leaves in 50% shading)	0.19[ab]
N2D1 (young leaves in non-shading)	0.14[b]
N2D2 (older leaves in non-shading)	0.24[a]

Numbers followed by the same letter in the same column are not significantly different with DMRT test, α=5. *Means of three replications

carbon to synthesise secondary metabolites as defence mechanism. Ascorbic acid is synthesised by using carbon such as D-galactose or D-glucose as a precursor (Loewus, 1999). It has an important role as co-factor in several enzymes and functions as antioxidant that protects photosynthetic apparatus from high light intensity (Pignocchi & Foyer, 2003; Smirnoff, 1996).

Results showed that ascorbic acid is more responsive than oleuropein in different environmental conditions, particularly when exposed to sunlight. It is assumed that under full exposure to sunlight, the synthesis of ascorbic acid will be increased to reduce the detrimental risk caused by ROS. According to Gallie (2013), ascorbic acid can detoxify free radicals as a result of full exposure to the sun. High light intensity can increase the formation of mono oxygen that can cause damage to membrane and pigments in PSI and PSII.

The ascorbic acid concentration in older leaves when exposed to sunlight (N2D2) was higher; this indicates that in olive plants, the defence mechanism through ascorbic acid is more efficient in older leaves than in younger ones. It is supported by the oleuropein content which is also higher in older leaves.

Olive tea leaves can be part of a healthy diet due to their high oleuropein content. Additionally, the tea leaves have ascorbic acid at the level of 85 mg per 100 g or 0.085% in average (data not presented). The awareness of the importance of ascorbic acid to human nutrition has facilitated the development of technologies that increase the ascorbic acid content of plants through manipulation of the biosynthetic or recycling pathways (Gallie, 2013).

CONCLUSION

Partial shading (50% shade) improved plant growth . Use of commercial compost resulted in more leaves and branches. Partial shading and compost encouraged the growth of leaves which are a good source of antioxidants. Oleuropein content in dried leaves and in fresh leaves was relatively constant, regardless of shade and the type of fertiliser used. Older leaves contained higher amount of oleuropein and ascorbic acid than the younger ones. It is suggested that under climatic conditions in the Western part of Indonesia (constant temperature over the year) where mix-farming is also commonly practised, olive trees can grow well and the leaves containing high levels of antioxidants.

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