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The influence of olive tree fertilization on the phenols in virgin olive oils. A review

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SUMMARY: The total phenols in virgin olive oil are highly dependent on cultivar, but also on ripening stage and other agronomic factors. The focus of most studies on agronomic factors has been irrigation, while fertilization has received less attention. Most of the fertilization works find that nitrogen over-fertilization leads to a decrease in phenol contents in virgin olive oil (VOO) and extra virgin olive oil (EVOO), under rain-fed or irrigation management. Ortho-diphenols also decrease with high doses of nitrogen, with no effect on secoiridoids. Phosphorous has a minor effect on irrigated trees; while the role of potassium is controversial, with a lack of trials with calcium and micro-nutrients. Due to the great impact of the fertilization on the phenol content and quality of VOO, new research is necessary with focus aimed at different cultivars and agronomic factors.

KEYWORDS: *Nitrogen; Nutrients; Ortho-diphenols; Phenols; Secoiridoids*

RESUMEN: ***Influencia de la fertilización del olivo en los fenoles del aceite de oliva virgen. Revisión.*** Los fenoles del aceite de oliva tienen un fuerte componente varietal, estando influenciados por el estado de maduración y otros factores agronómicos. Entre estos últimos, el efecto del riego es el más conocido, mientras que la fertilización está menos estudiada. La mayoría de los trabajos existentes sobre fertilización, muestran que el incremento en el abonado nitrogenado disminuye los niveles de fenoles totales del aceite de oliva virgen o virgen extra, tanto en secano como en regadío. También se han observado niveles inferiores de ortodifenoles en tratamientos con aplicación de nitrógeno, sin detectar diferencias en el contenido de secoiridoides. El fósforo tiene una influencia menor y la del potasio es algo controvertida, quedando por explorar la influencia del calcio y de los micronutrientes. Debido al considerable impacto que tiene la fertilización en los fenoles así como en la calidad del aceite, sería necesario incrementar los estudios de fertilización con diferentes variedades y sistemas productivos.

PALABRAS CLAVE: *Fenoles; Nitrógeno; Nutrientes; Ortodifenoles; Secoiridoides*

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1. INTRODUCTION

Due to its present and future strategic value, olive production systems for the production of olive oil have received a strong push in terms of technological innovation in the last twenty years. A higher number of plants per hectare, mechanized harvesting and the use of fertigation are some of the innovations that involve what is called agricultural intensification. The intensification of olive orchard management implies an increase in irrigation and fertilization, especially nitrogen (N), which can lead to a reduction in virgin olive oil's (VOO[1]) quality and stability, in particular, a decrease in phenol content. There is a negative relationship between irrigation and phenol content, as different authors have demonstrated in studies with linear irrigations, where the total applied irrigation water linearly changed with the effective crop coefficient (Tovar *et al.,* 2001; Berenguer *et al.,* 2006) or deficit irrigations (Gómez-del-Campo, 2013; Rufat *et al.,* 2018), although no differences were found in intermediate irrigation strategies (Sastre *et al.,* 2019), which linearly increased the total amount of applied water. Berenguer *et al.* (2006) found differences among the most contrasting treatments. Phenols are highly influenced by cultivar and ripening stage (Aguilera *et al.,* 2017), and also by other agronomic factors such as harvesting method (Morales-Sillero and García*,* 2015; Arbonés *et al.,* 2016).

Water availability is important for determining plant response to mineral nutrition (Marschner, 2012) at both the irrigation level and N status, which are highly related to the cultivar. At present, the question stated by Zipori *et al.* (2015) should be answered "Can threshold values being used today be applied indiscriminately to all cultivars, especially in intensive, irrigated orchards?"

The influence of ripeness and irrigation on the phenol content in VOOs phenols has been studied in recent years (Aparicio and Harwood, 2013; Mena *et al*., 2018; Vidal *et al*., 2019), although there are few works related to fertilization (Lanza and Ninfali, 2020; Zipori *et al*., 2020) despite its crucial role in VOO's quality. Fertilization should be carefully monitored to avoid negative impacts on VOO (Rallo *et al*., 2018). This work aims to review existing studies and research related to the influence of olive orchard fertilization on the phenols in VOO.

(1) VOO includes Extra Virgin and Virgin Olive Oil categories as are described in the Regulation (EU) No 1308/2013 (EU, 2013).

2. NITROGEN, PHOSPHORUS AND POTASSIUM INFLUENCE ON VOO PHENOLS

Despite the fact that most of the studies show that an increase in N fertilization leads to a decrease in the phenol content of the VOOs in a different way, depending on olive orchard management and cultivars, some results are not so clear (Inglese *et al.,* 2002; Mezghani *et al.,* 2018).

In a long-term trial under rainfed conditions, Fernández-Escobar *et al.* (2002) found that increasing N doses up to 1 kg N·tree^{-1} resulted in a decrease in total phenols in VOO with no increase in yield or vegetative growth, despite higher values in leaf and fruit N content compared to the control. This occurred regardless of the plantation system (Fernandez-Escobar *et al.,* 2006). Moreover, foliar applications of B and N in Koroneiki induced a phenol decrease but a foliar algae solution (derived from fronds and strap-like blades of the seaweed *Ascophyllum nodosum* L. and applied at a concentration of 0.5%) did not (Chouliaras *et al.,* 2009).

A decrease in total phenol content was observed in response to high doses of N and phosphorus (P) while potassium (K) had only a minor effect or no effect at all on irrigated trees (Dag *et al.,* 2009; Lanza and Ninfali*,* 2020), while Pascual *et al.* (2019) found a significant decrease with K fertilization. Erel *et al*. (2013), who observed a decreased in phenol content as foliar N increased, found the same trend. They also found a weaker relationship between phenol content and fruit N, without finding a correlation between phenol content and fruit P content. In the Barnea cultivar, Dag *et al.* (2018) observed a reduction in total phenol content when high N doses were applied, similar to what Morales-Sillero *et al.* (2007) observed in the Manzanilla de Sevilla cultivar, although significant differences with fruit N content were not found. Phosphorous enhances the accumulation of N in leaves and fruits and thus indirectly increases the nutritional level of N. Total phenol concentration was entirely independent of the leaf or fruit K levels, and therefore the effect of the N on oil properties depended solely on the absolute N content in the leaves or fruits, regardless of the cause. Olive trees can deal with a low soil fertility unless a great deficiency occurs, but an overdose in N, P or K fertilization can lead to a reduction in VOO quality (Tognetti *et al.,* 2008).

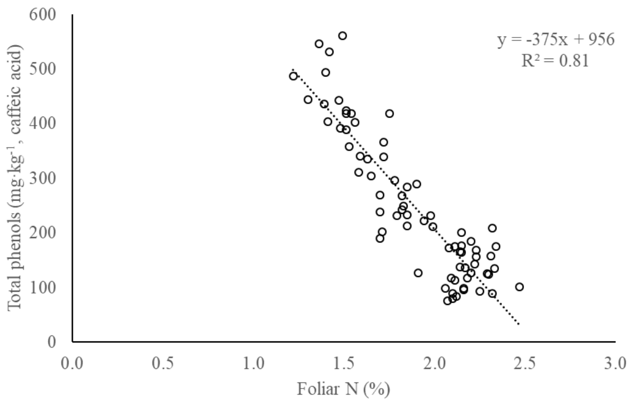

FIGURE 1. Linear regression between leaf N content and total phenols (R^2 = 0.806) in the VOO of cv Arbequina from two super-intensive orchards in Madrid and Lleida (Spain) with different levels of irrigation and fertilization with nitrogen and potassium. N=64.

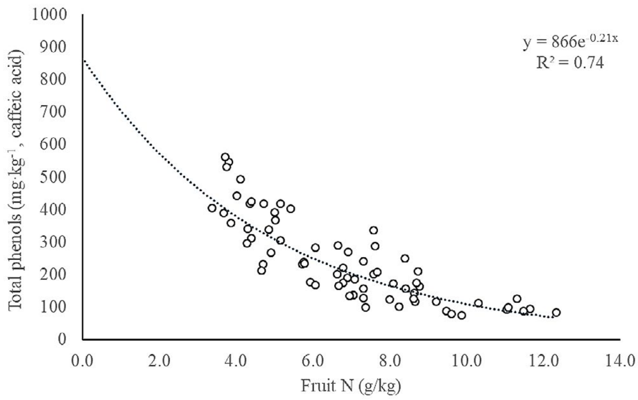

FIGURE 2. Quadratic regression between fruit N content and total phenols (R^2 = 0.743) in the VOO of cv Arbequina from two super-intensive orchards in Madrid and Lleida (Spain) with different levels of irrigation and fertilization with nitrogen and potassium. N=60.

Arbonés *et al.* (2017) also found a negative correlation between total phenols and N foliar content in a trial with N and K fertilization in super-intensive hedgerows of the Arbequina cultivar in Lleida (Spain). Only in ON years (heavy yield) did K application have a positive response, and a N/K interaction was evidenced by the effect of K on increasing leaf N content. Moreover, a high decrease in total phenols with K fertilization was found (Pascual *et al.*, 2019). They also established a critical nutrient range for N leaf in the interval of 1.80–1.94% with no effect on VOO yield, while beyond this threshold, phenol content and oxidative stability were compromised. That statement was confirmed by Arbonés *et al.* (2020), with cv Arbequina in two super-intensive orchards in Madrid and Lleida (Spain) with different levels of irrigation and N-K fertilization, finding a good negative linear correlation (R^2=0.81) between foliar N and total phenol content in VOO (Figure 1). Beyond 1.9% leaf N, the total phenol content decreased to below 200 $mg \cdot kg^{-1}$ (expressed as caffeic acid), a value considered to be the threshold above which phenolic compounds exert their nutraceutical effects as antioxidants (European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies, 2011; Gucci *et al.*, 2012). This fact gives rise to a reduction in VOO quality. The relationship between fruit N (pulp plus stone) and total phenol content is quadratic, and it is highly influenced by crop load (Figure 2). Below 7 $g \cdot kg^{-1}$ fruit N, VOO's phenol content falls below the 200 $mg \cdot kg^{-1}$ (expressed as caffeic acid).

Within the phenol family, increasing foliar and fruit N levels gives rise to a decrease in total oil phenols (around 250 mg per N foliar unit), specially in secoroid compounds, which results in lower VOO quality and stability. This negative impact of N on VOO quality is higher in OFF years (low yield). These results differ from those of Centeno *et al.* (2017) with cv Arbequina, who observed lower ortho-diphenol (o-diphenols) contents in all N-fertilized treatments regardless of doses or products, whilst total secoiridoids were only reduced in treatments with N inhibitors. In Picholine cultivar under irrigation conditions, Tekaya *et al.* (2014) found a significant decrease in total phenols with urea and micronutrient fertilization. The same group did not find a negative effect of N fertilization on total phenols but they observed a great decrease in o-diphenol content (Tekaya *et al.,* 2013).

Nitrogen is one of the nutrients that can modulate the activity of different enzymes related to phenol content and metabolism, like phenylalanine ammonium lyase (PAL) and polyphenol oxidase (PPO). In different plants (e.g. basil, tobacco and olive tree), PAL activity, involved in the synthesis of phenols, can be negatively influenced by N fertilizacion due to a competition between phenols and protein in fruit (Ruiz *et al.,* 1998; Fernández-Escobar, 2011; Jakovljević *et al.*, 2019). Furthermore, PPO activity increases as foliar and fruit N contents increase, with a negative effect on VOO stability due to changes in its fatty acid profile (Pascual *et al.,* 2016).

The synthesis of phenolic compounds is stimulated in response to different plant stresses (Fernández 2014; Rallo *et al.,* 2018). Therefore, in intensive and super-intensive olive orchards, from the point of view of the quality of the VOO, it should be appropiate to exert a certain level of stress, but not too high so as to reduce crop load.

3. OTHER NUTRIENTS THAT INFLUENCE VOO PHENOLS

A decrease in total phenols and o-diphenols has been observed after foliar fertilization with boron (B), magnesium (Mg), manganese (Mn) and sulfur (S), due to the B action (Tekaya *et al.,* 2013). When the content of B in the leaves is low, phenolic biosynthesis increases, but when the level of boron is adequate, the PAL activity is depressed and phenol biosynthesis is limited (Ruiz *et al.,* 1998). Under semiarid conditions, Saadati *et al.* (2013) did not find statistical differences in total phenols for different cultivars after applying boric acid and zinc sulfate. Increases in VOO oil content and phenolic compounds were observed when cv. Beledy olive trees were treated with Zn in the form of amino acid chelate (Bastam *et al.,* 2020). In the Chemlali cultivar, the foliar spraying of amino acids, beet molasses and fish by-products as sources of N, P and K resulted in a decrease in the phenol content, contrary to an application of a formulation based on calcium (Ca), which led to a significant increase in phenolic compound contents (Dabbaghi *et al.,* 2019).

Foliar fertilization with selenium (Se) applied to the Leccino cultivar increased the total Se, alleviated damage caused by drought stress conditions and increased total antioxidant compounds, mainly phenol contents (D'Amato *et al.,* 2018). Silicon (Si), like selenium, is a non-essential element in plant nutrition despite the fact that it plays an important role against drought and other abiotic stresses (Marschner 2012). Different studies have found that Si forms a physical barrier and induces the production of phenolic compounds at sites of infection (Fernandez-Escobar, 2019). Silicon applying can be done through drip irrigation or the most effective foliar spraying, which can be promising for olive orchards, mainly under rainfed conditions.

4. CONCLUSIONS

Nitrogen (N) over-fertilization leads to a total phenol content decrease, regardless of the olive growing system or cultivar type; phosphorus fertilization seems to have a minor influence, and in the case of potassium (K), the effect is controversial.

Olive yield decreases beyond a threshold of foliar N, for this reason it is essential to establish the proper reference levels, which do not impair olive yield whilst maintaining a sufficient phenol content to maintain good VOO quality and stability. This threshold should be different for each cultivar and olive-growing system.

Phosphorus fertilization seems to have an indirect influence on phenol content, although results on the effect of K differ among authors. There is a lack of field trials with these macro-nutrients near or below the deficiency threshold to clarify the role of K regarding phenol content. New trials with potassium-based formulations could be of great interest, in order to increase total phenols in cultivars with low to medium phenol content.

Fertilization with micronutrients and other non-essential microelements have been scarcely studied. These elements must be considered for future studies, mainly boron and selenium elements, which could produce an increase in total phenols, or silicon, which could protect fruit skin, thus avoiding the loss of phenols during mechanical harvesting.

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