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# The Use of Biostimulants in High-density Olive Growing: Quality and Production

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors GHH and ILC designed the study and performed the statistical analysis, authors DMS and ILC wrote the protocol and wrote the first draft of the manuscript. Authors GHH and JMT managed the analyses of the study. Authors JMT and DMS managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

Due to the increase of high-density holdings, especially of olive trees, the nutritional requirements of the plants are higher per unit area, which implies that a greater contribution of fertilizers to the soil is needed. Opting for fertilizers of inorganic origin will produce an increase in the pollution of the soil.

In the face of this possible soil contamination, our aim is to analyze the effect of biostimulants as an alternative to chemical fertilizers, to steadily produce and maintain high quality standards during the life of the crop. Our objective is using more environmentally friendly products in order to satisfy one of the most important demands from both consumers and the authorities.

In this study, we carried out five different treatments in addition to a control treatment with a supply of NPK, from inorganic products, which are used to control fertilization with a solution obtained from seaweed extracts. These treatments were applied in two crop cycles for two of the most important varieties in the current olive tree growing scenario: Arbequina and Koroneiki.

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This study was developed in the farm *Pozohondo,* which is located in a crop zone by the *Palancia* river (Castellón, Valencia, Spain), in the southeast of the Iberian Peninsula, where the olive trees were established in a high-density system with a planting framework of 4 x 1.5 m. We ensured an exhaustive control of the nutritional needs of the holding by using a fertigation system.
We could notice differences in the productions of each applied treatment, avoiding any possible biases through the additional control of 100 randomly selected olives from each of the samples. There is an improvement in the set of physical characteristics of the olives with the treatment that provides amino acids and extra potassium based on amni acids. We analyzed the quality of the olive oil obtained from the production of each treatment by measuring the fatty acids, tocopherols and polyphenols contents. We also carried out an organoleptic tasting analysis following the rules of the International Olive Committee (IOC).
We observed an improvement with regard to the rest of treatments in the pomological parameters of the olives when applying the potassium and amino acid biostimulant, while the quality of the oils was not affected by the type of fertilization applied in each treatment.

*Keywords: Biostimulant; NPK; olive growing; chemical fertilizers.*

## 1. INTRODUCTION

The olive tree is a traditional growing throughout the Mediterranean Basin and it plays a key role in the so-called Mediterranean Diet [1]. At present, the surface cultivated in Spain of olive grove is 2.697.445 hectares, which supposes more than 50 % of the surface devoted to the cultivation of woody species in this country. Its oil is said to have nutraceutical properties, mainly due to its monounsaturated fatty acids, polyphenols and tocopherols contents, which provide antioxidant, antimicrobial and carcinogenic activities, among others [2].

There is a clear tendency nowadays towards the use of environmentally friendly cropping techniques, there is a special interest in the practice of organic fertilization with products coming from extracts of algae and/or crops, which provide a high organic matter content that delivers the necessary nutrients to the plant.

It is well documented that a suitable irrigation regime increases the size and weight of the olives, in addition to improving the pulp/endocarp relation [3], the difference is greater when a custom fertilization is applied [4]. The use of fertilizers exceeds 100 billion kilograms per year. This value has increased steadily in recent years, along with the introduction of growings in high-density systems, which increase fertilizer consumption and can lead to overuse contamination [5] producing salinization and sodification of soils [6].

In general, biostimulants have been described as products that contain substances and/or microorganisms whose function is to stimulate natural processes, to enhance nutrient uptake, and to improve nutrient use efficiency, tolerance to abiotic stress, and crop quality when applied to plants or the rhizosphere. Council [7]. According to Chen et al. [8] this type of compounds enhance soil microbial activity, thereby improving the fungal and bacterial activity in the long term, as well as improving the crop itself.

Algae extracts are one of the most important components in the composition of biostimulants. They enhance plant development and are beneficial for both human and animal health [9]. The major components of commercial SWE are polysaccharides, followed by phenolics, vitamins precursors, osmolytes (mannitol), phytohormones, and hormone-like compounds [10]. Furthermore, they improve plant resistance to both biotic and abiotic stress [11], activating different metabolites that provide the plant with a better defense against pathogens [12].

The market for biostimulants has not stopped growing since the year 2013, at the rate of about 12% per year, and their main destinations are European holdings, which meant more than 6 million hectares in our continent that year [13].

In addition to improving plant development, biostimulants increase the biomass in different crops such as the almond tree [14]. Another type of products that falls within the definition of biostimulants, such as compost, improves the development and growth of peach trees [15]. On the other hand, it is important to point out that there is not only an increase in the productions, but also an organoleptic improvement of production in the case of fruit trees [16].

Some studies consider that fertilization has no effect on the organoleptic characteristics of the

product obtained, however, it can alter the composition of compounds such as polyphenols in olive oils [17].

It has also been written that products grown in more environment-friendly conditions are tastier [4], on the other hand, oils show a higher content of monounsaturated fatty acids [18]. People have been proven to have a greater interest in pesticide-free products that present some type of certification, such as ecological or organic products [19], so it is interesting to carry out studies in this area.

Each cultivar possesses singular characteristics in composition of virgin olive oil [20], but the location and climatic conditions also influence in this characteristics [21].

The Arbequina variety is known for adapting to high density cultivation, it is a Spanish origin cultivar whose fruits are small and round and its oils are smooth only slightly bitter and peppery.

The Koroneiki is a Greek origin variety, it is very important in the production of oils. It provides an intense green color which is very much appreciated by consumers. Its fruits are large and oval, the oils obtained from its olives have a bitter are peppery taste, as opposed to the Arbequina cultivar.

Despite all the benefits involved in the use of this type of products (biostimulant fertilizing treatments), it is necessary to understand that carrying out a fertilization process of this type is a complex activity that requires meeting the nutritional needs of the plant as well as ensuring soil fertility [22]. That is why this study aims to evaluate the possible production and quality differences in an intensive cultivation of olive trees by comparing biostimulant fertilizing treatments in order to prove if it is possible to maintain the productive performance of an intensive system holding, using environmentally friendly fertilization. Our study focuses on the search for an environmentally friendly fertilization as well as the achievement of an optimum production while maintaining the highest standards of both chemical and organoleptic qualities.

## 2. MATERIALS AND METHODS

The study was carried out in an olive tree exploitation located in the province of Castellón (Spain) (39°53'50.1" N 0°31'28.0" W) in the southeast of the Iberian Peninsula, in an area with an average temperature of 14.2°C and an average annual rainfall of 384 mm per year. The planting pattern was 1.50 x 4.00 meters, for trees of two of the main varieties that are used in this type of growings, the Arbequina and the Koroneiki varieties, that are 2.50 meters high, 20 years of age, and in full production. The plot had a fertigation system with which the contributions of irrigation and fertilization were made, irrigation was 3500 m3 per hectare per year, distributed throughout the periods when the cultivation needed the most water, from June, when olives are in BBCH 69 state (end of the flowering and ripening of the fruit), until mid-September, when the trees are in BBCH 89 state (the fruits acquire the characteristic color of their variety, they remain turgid. Fruits are suitable for the extraction of the oil).

The biostimulants were tested in an Arbequina and Koroneiki cultivar tree holding, given their importance in the current olive growing, and more specifically in high-density cultivation systems.

Each of the cultivars had 5 different fertilizing treatments, in addition to a control treatment with fertilizer NPK (T0). The composition of each of the products applied in each treatment can be seen in Table 1. The treatments applied were T1 (potassium fertilization), T2 (fertilization with

**Table 1. Composition of applied treatments[o]**

| Treatment | Composition |
|---|---|
| T0 | NPK-based fertilization (130 UF N, 35 UF $P_2O_5$, 180 UF $K_2O$) |
| T1 | Potassium fertilization (60 % $K_2O$) |
| T2 | Fertilization with seaweed-based biostimulant (2,08 % Bo, 0,02 % Mo and GA142 seaweed filtrate) |
| T3 | Potassium nitrate based fertilization (60 % $NO_3$ + 38 % $K_2O$) |
| T4 | Potassium and algae-based biostimulant fertilization (60 % $K_2O$) + (2,08 % Bo, 0,02 % Mo and GA142 seaweed filtrate) |
| T5 | Potassium fertilization and amino acid based biostimulant (60 % $K_2O$) + (12 % Aminoácidos libres + 8,5 % N + 2,5 % MgO) |

seaweed-based biostimulant, whose main ingredients are Boron and Molybdenum), T3 (potassium nitrate based fertilization), T4 (potassium and algae-based biostimulant fertilization) and T5 (potassium fertilization and amino acid based biostimulant. Amino acids were composed mainly of free amino acids, nitrogen and manganese oxide).

In order to calculate the production of the trees, fruit from 4 randomly selected trees per treatment and cultivate was collected manually. To this effect, 2 trees of each of the rows treated with each treatment were selected, avoiding the trees at the beginning and the end of the rows that might be affected by passing vehicles.

The first step taken to analyze the olives was characterizing them pomologically following norm UPOV-CPVO (Union for the Protection of Variety Obtention) of the olive tree TG/99/4, as a system to establish a pomological characterization of the olive material to be used in the study.

Once the pomological analysis was carried out, we conducted a pomometric analysis of the olives by measuring the weight, length, width A and width B of each of them, after which we proceeded to the study of the endocarps, and at the same time obtained the pulp/endocarp relation.

In a pilot plant installation, we proceeded to obtain the oil production from each of the samples. These olives were crushed in a hammer mill in order to obtain the olive mass that was then poured into a blender in a bath to keep the temperature below 21°C and thus extract the individual oil in each of the fertigation trials. After this process was completed, the mass was then centrifuged to separate the oil from the solid and aqueous phase obtained after the blending phase.

Once the oils were separated, a sample of each of them was taken to be analyzed in the laboratory, in order to get the parameters that indicate their quality from a chemical point of view by analyzing the polyphenols, tocopherols and fatty acids contents. This process was aimed at verifying that they were extra virgin olive oils (EVOO), complying with the highest standards of quality as well as obtaining a complete chemical characterization. An organoleptic analysis through tasting was carried out on the rest of the sample, in accordance with the rules of the International Olive Oil Council (IOOC) [23].

In order to determine the fatty acid composition of the olive oil a sample was subjected to transesterification with methanolic potassium hydroxide and n-heptane. The following fatty acids were determined: palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), eicosanoic–arachidic acid (C20:0), docosanoic–behemic acid (C22:0), and tetracosanoic–lignoceric acid (C24:0).

Three sterols were examined: β-sitosterol, stigmasterol and campesterol. The oil sample was saponified with an ethanolic potassium hydroxide solution. The unsaponifiable fraction was removed with an ethyl ether. The unsaponifiable sterol fraction was separated by silica gel plate chromatography. Separation and quantification of the silanized sterol fraction was carried out by means of a capillary column in a gas chromatograph, Hewlett-Packard model HP 5840 gas chromatograph, equipped with an FID-300, which worked at 290°C. The sample was injected at 280°C, following an isothermal process at 265°C for 45 min using a HP-5MS capillary column (30 m × 0.25 mm × 0.22 μm). This column was filled with film OB5 Tracer-Tecnocroma. The working conditions were as follows: Helium flow was 1 mL/min; the injector temperature was 300°C; and the detector temperature was 290°C. The injection volume was 0.2 mL at a flow rate of 1.1 mL/min (Commission Regulation (EEC) No. 2568/91, corresponding to AOCS method Ch 6–91). The compounds were quantified by addition of an internal pattern (5-α-cholestanol). The sterol concentration was expressed as mg/100 g of fatty matter. The area of peaks generated by the sterols was carried out by an automatic integrator.

α-Tocopherol was evaluated following AOCS method Ce 8–89. A solution of oil in hexane was analyzed on an Agilent Technologies HPLC system (1100 series) on a silica gel Lichrosorb Si-60 column (particle size 5 μm × 250 mm × 4 mm i.d. of Sugerlabor, Madrid, Spain) using n-hexane/2-propanol (98.5/1.5, vol/vol) at a flow rate of 1 mL/min. A fluorescence detector (Thermo-Finnigan FL3000) was used, with

excitation and emission wavelengths set at 290 and 330 nm, respectively.

We used the program Statgraphics Centurion XVII for the statistical analysis, performing variance analysis (ANOVAs) with a 95% significance to analyze each of the parameters individually.

## 3. RESULTS AND DISCUSSION

In the pomometric characterization of the Arbequina variety, we observed differences between the studied treatments in the size and weight of the fruits and their endocarps, as reflected in Table 2. In the two studied campaigns, we could observe that the heaviest fruits were the ones who had received an extra intake of potassium and amino acids biostimulant (T5), with an average weight between 1.30 and 1.38 grams in each campaign, while the lighter fruits were the control treatment with a weight between 0.92 and 0.93 grams in each campaign, this has an impact on the pulp endocarp that usually marks the performance of the fruits, so it is one of the most relevant values that are generally studied. Thus, the treatments that represented the maximum and minimum values for this parameter were repeated, and the fruits with a higher pulp/endocarp relation, ranging between 76 % and 78 %, came from trees treated with an extra supply of amino acids and potassium (T5), while the fruits of the control treatment that received conventional NPK fertilization, recorded a lower pulp/endocarp relation of between 65% and 70%, just like Laila et al. [24], in our study, we improved the caliber of the olives with biofertilizer contributions.

In the case of the Koroneiki variety fruits, the differences between treatments were lower than in the Arbequina variety, even so, in the two campaigns in study, we observed an improvement in the size and the pulp/endocarp relation in the fruits treated with an extra supply of potassium and amino acid biostimulant (T5) with respect to the rest of the treatments. The average weight of the fruits collected in the trees that received this treatment was between 0.75 and 0.80 grams.

On the other hand, the treatment with lighter fruits and less pulp/endocarp relation was the control treatment. Chouliaras et al. [25] obtained an improvement in the pomometry of the fruits of this variety when applying algae extract biostimulants, similar to our T2 treatment, while those who had lower values for the pomometric parameters in study were those in the control treatment, with a fruit weight between 0.45 and 0.54 grams, which is reflected in Table 3, where the pulp/endocarp relation of the fruits under the T5 treatment (extra supply of potassium and amino acid based biostimulant) presented an average value in both campaigns of 73%, whereas in the control treatment, they varied between 61% and 64%.

With regard to the productions per tree, the same applies for the pomometry, trees that showed a better performance, and therefore increased production during the two campaigns of cultivation under study, were those belonging to the crop lines treated with an extra supply of potassium and amino acid biostimulant (T5) for both varieties. There was an average production of 6.35 kg per tree in the trees of the Arberquina variety in which this treatment was applied, while the trees in the control treatment barely achieved an average production of 4.87 kg per tree. On the other hand, in the Koroneiki variety, production was 7.45 kg per tree in the lines treated with an extra supply of potassium and amino acid biostimulant (T5), while the trees of the control treatment lines obtained an average production of 4.8 kg per tree.

After analyzing the composition of the obtained oils, as shown in Tables 4 and 5, we found that the fatty acids, polyphenols and tocopherols contents were not significantly affected in any of the various combinations variety-treatment, there were only small variations in some of them. However, other authors such as Tekaya et al. [26] have seen significant variations in the content of tocopherols. This may be due to the fact that our study was conducted in a high density growing which was not the case in the studies of these authors.

When carrying out the organoleptic characterization of the oils obtained for each variety-treatment combination, we proved that none of the treatments applied had altered the characteristics of the monovarietal oils of the varieties under study. So there has been no differences between the values obtained from each of the flavours appreciated by this method. This allows to establish that, in the use of biostimulants, organoleptic conditions remain unchanged and will continue to be of interest to consumers who are used to these varietal features.

**Table 2. Fruit pomometric characterization of cultivar Arbequina**

| | Cultivar arbequina first year | | | | | |
|---|---|---|---|---|---|---|
| | **T0** | **T1** | **T2** | **T3** | **T4** | **T5** |
| Fruit weight (g) | 0.93 ± 0.19$^{e}$ | 1.14 ± 0.22$^{c}$ | 0.99 ± 0.29$^{d}$ | 1.03 ± 0.27$^{d}$ | 1.21 ± 0.23$^{b}$ | 1.38 ± 0.24$^{a}$ |
| Fruit length (mm) | 12.41 ± 1.14$^{d}$ | 13.49 ± 1.12$^{b}$ | 12.66 ± 1.25$^{c}$ | 13.36 ± 1.16$^{b}$ | 13.46 ± 1.02$^{b}$ | 14.56 ± 1.11$^{a}$ |
| Fruit width A (mm) | 10.60 ± 0.93$^{d}$ | 11.51 ± 0.86$^{b}$ | 10.67 ± 1.09$^{d}$ | 11.13 ± 1.02$^{c}$ | 11.51 ± 0.93$^{b}$ | 12.80 ± 0.88$^{a}$ |
| Fruit width B (mm) | 10.20 ± 0.84$^{d}$ | 11.13 ± 0.83$^{b}$ | 10.19 ± 1.08$^{d}$ | 10.77 ± 1.02$^{c}$ | 11.09 ± 0.86$^{b}$ | 12.39 ± 0.87$^{a}$ |
| Endocarp weight (g) | 0.27 ± 0.06$^{d}$ | 0.30 ± 0.06$^{b}$ | 0.29 ± 0.07$^{cd}$ | 0.30 ± 0.06$^{b}$ | 0.33 ± 0.06$^{a}$ | 0.30 ± 0.05$^{bc}$ |
| Endocarp length (mm) | 9.33 ± 0.81$^{d}$ | 10.05 ± 0.85$^{b}$ | 9.76 ± 0.94$^{c}$ | 9.92 ± 0.84$^{bc}$ | 10.36 ± 0.82$^{a}$ | 10.14 ± 0.80$^{ab}$ |
| Endocarp width A (mm) | 6.63 ± 0.48$^{d}$ | 6.82 ± 0.48$^{b}$ | 6.75 ± 0.50$^{bc}$ | 6.70 ± 0.47$^{cd}$ | 7.03 ± 0.46$^{a}$ | 6.66 ± 0.35$^{cd}$ |
| Endocarp width B (mm) | 6.45 ± 0.46$^{d}$ | 6.64 ± 0.43$^{b}$ | 6.57 ± 0.52$^{bc}$ | 6.50 ± 0.44$^{cd}$ | 6.82 ± 0.42$^{a}$ | 6.52 ± 0.36$^{cd}$ |
| Pulp/endocarp relation | 0.70 ± 0.04$^{c}$ | 0.74 ± 0.04$^{b}$ | 0.70 ± 0.05$^{c}$ | 0.70 ± 0.04$^{c}$ | 0.73 ± 0.03$^{b}$ | 0.78 ± 0.03$^{a}$ |
| | **Cultivar Arbequina second year** | | | | | |
| | **T0** | **T1** | **T2** | **T3** | **T4** | **T5** |
| Fruit weight (g) | 0.92 ± 0.21$^{d}$ | 0.96 ± 0.32$^{d}$ | 1.06 ± 0.20$^{b}$ | 1.00 ± 0.21$^{c}$ | 1.03 ± 0.23$^{bc}$ | 1.30 ± 0.30$^{a}$ |
| Fruit length (mm) | 12.93 ± 0.97$^{d}$ | 12.50 ± 1.30$^{d}$ | 13.03 ± 1.13$^{b}$ | 13.41 ± 1.27$^{bc}$ | 12.89 ± 1.18$^{c}$ | 13.91 ± 1.15$^{a}$ |
| Fruit width A (mm) | 10.44 ± 0.86$^{c}$ | 10.32 ± 1.18$^{d}$ | 11.12 ± 0.85$^{c}$ | 10.96 ± 0.83$^{b}$ | 10.84 ± 0.97$^{c}$ | 12.01 ± 1.06$^{a}$ |
| Fruit width B (mm) | 10.11 ± 0.82$^{d}$ | 10.00 ± 1.15$^{d}$ | 10.72 ± 0.82$^{b}$ | 10.79 ± 0.91$^{b}$ | 10.49 ± 0.92$^{c}$ | 11.75 ± 1.05$^{a}$ |
| Endocarp weight (g) | 0.31 ± 0.06$^{b}$ | 0.28 ± 0.06$^{c}$ | 0.34 ± 0.08$^{a}$ | 0.32 ± 0.07$^{b}$ | 0.31 ± 0.07$^{b}$ | 0.31 ± 0.06$^{b}$ |
| Endocarp length (mm) | 10.27 ± 0.97$^{b}$ | 9.92 ± 0.92$^{c}$ | 10.49 ± 1.09$^{a}$ | 10.17 ± 1.04$^{b}$ | 10.19 ± 0.86$^{b}$ | 10.16 ± 0.88$^{b}$ |
| Endocarp width A (mm) | 6.87 ± 0.50$^{bc}$ | 6.72 ± 0.51$^{e}$ | 7.31 ± 0.71$^{a}$ | 6.93 ± 0.65$^{b}$ | 6.77 ± 0.51$^{de}$ | 6.83 ± 0.51$^{cd}$ |
| Endocarp width B (mm) | 6.66 ± 0.44$^{bc}$ | 6.53 ± 0.48$^{d}$ | 6.97 ± 0.63$^{a}$ | 6.69 ± 0.58$^{b}$ | 6.60 ± 0.48$^{cd}$ | 6.64 ± 0.48$^{bc}$ |
| Pulp/endocarp relation | 0.65 ± 0.08$^{d}$ | 0.69 ± 0.08$^{b}$ | 0.67 ± 0.06$^{c}$ | 0.65 ± 0.06$^{d}$ | 0.69 ± 0.08$^{b}$ | 0.76 ± 0.03$^{a}$ |

*T0 (NPK), T1 (potassium fertilization), T2 (fertilization with seaweed-based biostimulant), T3 (potassium nitrate based fertilization), T4 (potassium and algae-based biostimulant fertilization) and T5 (potassium fertilization and amino acid based biostimulant).Different letters indicate statistical significant differences in a 95%*

**Table 3. Fruit pomometric characterization of cultivar Koroneiki**

| Cultivar Koroneiki first year | | | | | | |
|---|---|---|---|---|---|---|
| | **T0** | **T1** | **T2** | **T3** | **T4** | **T5** |
| Fruit weight (g) | 0.54 ± 0.18$^{d}$ | 0.73 ± 0.13$^{b}$ | 0.69 ± 0.18$^{c}$ | 0.70 ± 0.13$^{bc}$ | 0.79 ± 0.18$^{a}$ | 0.80 ± 0.18$^{a}$ |
| Fruit length (mm) | 13.61 ± 1.11$^{d}$ | 14.79 ± 1.11$^{b}$ | 14.01 ± 1.64$^{c}$ | 15.04 ± 1.21$^{ab}$ | 15.12 ± 1.35$^{a}$ | 15.19 ± 1.44$^{a}$ |
| Fruit width A (mm) | 8.11 ± 0.89$^{e}$ | 9.01 ± 0.65$^{c}$ | 8.85 ± 0.83$^{d}$ | 9.27 ± 0.72$^{b}$ | 9.49 ± 0.73$^{a}$ | 9.52 ± 0.89$^{a}$ |
| Fruit width B (mm) | 7.81 ± 0.95$^{d}$ | 8.65 ± 0.64$^{bc}$ | 8.59 ± 0.81$^{c}$ | 8.78 ± 0.73$^{b}$ | 9.19 ± 0.75$^{a}$ | 9.13 ± 0.87$^{a}$ |
| Endocarp weight (g) | 0.18 ± 0.04$^{c}$ | 0.21 ± 0.04$^{b}$ | 0.19 ± 0.05$^{c}$ | 0.19 ± 0.04$^{c}$ | 0.22 ± 0.05$^{a}$ | 0.21 ± 0.04$^{b}$ |
| Endocarp length (mm) | 10.98 ± 1.05$^{c}$ | 11.66 ± 0.91$^{a}$ | 11.07 ± 1.24$^{c}$ | 11.37 ± 0.87$^{b}$ | 11.82 ± 1.06$^{a}$ | 11.75 ± 1.09$^{a}$ |
| Endocarp width A (mm) | 5.40 ± 0.36$^{c}$ | 5.62 ± 0.38$^{a}$ | 5.44 ± 0.38$^{bc}$ | 5.47 ± 0.32$^{b}$ | 5.58 ± 0.42$^{a}$ | 5.58 ± 0.35$^{a}$ |
| Endocarp width B (mm) | 5.29 ± 0.36$^{d}$ | 5.46 ± 0.37$^{a}$ | 5.33 ± 0.38$^{cd}$ | 5.37 ± 0.33$^{bc}$ | 5.43 ± 0.39$^{ab}$ | 5.47 ± 0.35$^{a}$ |
| Pulp/endocarp relation | 0.64 ± 0.07$^{d}$ | 0.71 ± 0.04$^{c}$ | 0.72 ± 0.04$^{bc}$ | 0.73 ± 0.03$^{ab}$ | 0.72 ± 0.04$^{c}$ | 0.73 ± 0.05$^{a}$ |
| **Cultivar Koroneiki second year** | | | | | | |
| | **Control** | **T1** | **T2** | **T3** | **T4** | **T5** |
| Fruit weight (g) | 0.45 ± 0.12$^{e}$ | 0.56 ± 0.09$^{d}$ | 0.55 ± 0.13$^{d}$ | 0.64 ± 0.11$^{c}$ | 0.67 ± 0.19$^{b}$ | 0.75 ± 0.13$^{a}$ |
| Fruit length (mm) | 13.23 ± 1.02$^{de}$ | 13.19 ± 0.89$^{e}$ | 13.34 ± 1.16$^{d}$ | 13.99 ± 1.03$^{c}$ | 14.43 ± 1.39$^{b}$ | 15.00 ± 1.29$^{a}$ |
| Fruit width A (mm) | 7.74 ± 0.60$^{e}$ | 8.35 ± 0.42$^{c}$ | 8.08 ± 0.75$^{d}$ | 8.73 ± 0.49$^{b}$ | 8.66 ± 0.91$^{b}$ | 9.21 ± 0.61$^{a}$ |
| Fruit width B (mm) | 7.48 ± 0.55$^{e}$ | 8.00 ± 0.42$^{c}$ | 7.77 ± 0.72$^{d}$ | 8.38 ± 0.49$^{b}$ | 8.41 ± 0.88$^{b}$ | 8.86 ± 0.61$^{a}$ |
| Endocarp weight (g) | 0.17 ± 0.03$^{d}$ | 0.17 ± 0.03$^{d}$ | 0.18 ± 0.03$^{c}$ | 0.18 ± 0.03$^{c}$ | 0.20 ± 0.04$^{a}$ | 0.20 ± 0.04$^{b}$ |
| Endocarp length (mm) | 10.69 ± 0.76$^{c}$ | 10.59 ± 0.75$^{c}$ | 10.86 ± 0.73$^{b}$ | 10.99 ± 0.86$^{b}$ | 11.40 ± 0.93$^{a}$ | 11.46 ± 1.03$^{a}$ |
| Endocarp width A (mm) | 5.23 ± 0.25$^{d}$ | 5.24 ± 0.29$^{d}$ | 5.30 ± 0.27$^{c}$ | 5.32 ± 0.32$^{c}$ | 5.49 ± 0.32$^{a}$ | 5.37 ± 0.36$^{b}$ |
| Endocarp width B (mm) | 5.11 ± 0.24$^{e}$ | 5.12 ± 0.28$^{de}$ | 5.17 ± 0.27$^{c}$ | 5.16 ± 0.30$^{cd}$ | 5.37 ± 0.31$^{a}$ | 5.25 ± 0.36$^{b}$ |
| Pulp/endocarp relation | 0.61 ± 0.08$^{e}$ | 0.69 ± 0.06$^{c}$ | 0.66 ± 0.07$^{d}$ | 0.72 ± 0.03$^{b}$ | 0.69 ± 0.06$^{c}$ | 0.73 ± 0.05$^{a}$ |

*T0 (NPK), T1 (potassium fertilization), T2 (fertilization with seaweed-based biostimulant), T3 (potassium nitrate based fertilization), T4 (potassium and algae-based biostimulant fertilization) and T5 (potassium fertilization and amino acid based biostimulant).Different letters indicate statistical significant differences in a 95%*

**Table 4. Olive oils fatty acids composition of the studied cultivars**

| Cultivar | Treatment | Fatty acids composition | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Miristic | Palmitic | Palmitoleic | Margaric | Margaroleic | Estearic | Oleic | Linoleic | Linollenic | Araquidic | Gadoleic | Behenic | Lignoceric |
| Arbequina | T0 | <0,01 | 11,01 | 1,19 | 0,06 | 0,08 | 2,27 | 78,60 | 5,31 | 0,58 | 0,42 | 0,31 | 0,15 | 0,02 |
| Arbequina | T1 | <0,01 | 10,97 | 1,12 | 0,08 | 0,10 | 2,38 | 78,53 | 5,38 | 0,56 | 0,41 | 0,34 | 0,11 | 0,02 |
| Arbequina | T2 | <0,01 | 10,88 | 1,10 | 0,05 | 0,08 | 2,35 | 78,72 | 5,32 | 0,57 | 0,44 | 0,30 | 0,15 | 0,04 |
| Arbequina | T3 | <0,01 | 10,98 | 1,15 | 0,05 | 0,09 | 2,34 | 78,55 | 5,37 | 0,54 | 0,44 | 0,33 | 0,14 | 0,02 |
| Arbequina | T4 | <0,01 | 11,12 | 1,18 | 0,04 | 0,10 | 2,36 | 78,69 | 4,96 | 0,60 | 0,43 | 0,35 | 0,14 | 0,03 |
| Arbequina | T5 | <0,01 | 11,02 | 1,16 | 0,04 | 0,11 | 2,35 | 78,94 | 4,86 | 0,57 | 0,42 | 0,35 | 0,15 | 0,03 |
| Koroneiki | T0 | <0,01 | 9,86 | 0,58 | 0,04 | 0,08 | 2,32 | 81,02 | 4,50 | 0,59 | 0,46 | 0,34 | 0,16 | 0,05 |
| Koroneiki | T1 | <0,01 | 9,88 | 0,59 | 0,04 | 0,07 | 2,35 | 81,01 | 4,51 | 0,63 | 0,44 | 0,30 | 0,14 | 0,04 |
| Koroneiki | T2 | <0,01 | 9,87 | 0,63 | 0,04 | 0,08 | 2,25 | 81,20 | 4,41 | 0,63 | 0,41 | 0,31 | 0,14 | 0,03 |
| Koroneiki | T3 | <0,01 | 9,88 | 0,61 | 0,04 | 0,07 | 2,38 | 80,81 | 4,68 | 0,65 | 0,42 | 0,29 | 0,13 | 0,04 |
| Koroneiki | T4 | <0,01 | 9,85 | 0,64 | 0,04 | 0,07 | 2,34 | 80,88 | 4,61 | 0,64 | 0,42 | 0,33 | 0,14 | 0,04 |
| Koroneiki | T5 | <0,01 | 9,82 | 0,59 | 0,05 | 0,07 | 2,36 | 80,97 | 4,59 | 0,62 | 0,44 | 0,31 | 0,14 | 0,04 |

*T0 (NPK), T1 (potassium fertilization), T2 (fertilization with seaweed-based biostimulant), T3 (potassium nitrate based fertilization), T4 (potassium and algae-based biostimulant fertilization) and T5 (potassium fertilization and amino acid based biostimulant)*

**Table 5. Tocopherols and Poliphenols content in olive oils of the studied cultivars**

| Cultivar | Treatment | Isomers trans | | Tocopherols/tocotrienols | | | | | Total poliphenols |
|---|---|---|---|---|---|---|---|---|---|
| | | Trans oleics | Tr L+Tr Ln | Total tocopherols | α-Tocopherol | β-Tocopherol | γ-Tocopherol | δ-Tocopherol | Poliphenoles (Cafeic) |
| Arbequina | T0 | <0,03 | <0,03 | 288,4 | 284,0 | 1,4 | 1,1 | <1 | 155 |
| Arbequina | T1 | <0,03 | <0,03 | 290,5 | 286,3 | 1,4 | 1,1 | <1 | 152 |
| Arbequina | T2 | <0,03 | <0,03 | 279,1 | 274,6 | 1,8 | 1,3 | <1 | 149 |
| Arbequina | T3 | <0,03 | <0,03 | 282,1 | 275,9 | 1,6 | 1,0 | <1 | 160 |
| Arbequina | T4 | <0,03 | <0,03 | 276,0 | 273,6 | 1,3 | 1,2 | <1 | 153 |
| Arbequina | T5 | <0,03 | <0,03 | 291,9 | 289,1 | 1,6 | 1,1 | <1 | 152 |
| Koroneiki | T0 | <0,03 | <0,03 | 239,8 | 228,8 | 2,0 | 3,6 | <1 | 174 |
| Koroneiki | T1 | <0,03 | <0,03 | 242,2 | 236,7 | 2,2 | 3,3 | <1 | 185 |
| Koroneiki | T2 | <0,03 | <0,03 | 236,4 | 231,3 | 2,1 | 3,1 | <1 | 175 |
| Koroneiki | T3 | <0,03 | <0,03 | 228,5 | 223 | 2,3 | 3,2 | <1 | 172 |
| Koroneiki | T4 | <0,03 | <0,03 | 249,5 | 243,3 | 2,3 | 4 | <1 | 165 |
| Koroneiki | T5 | <0,03 | <0,03 | 247,6 | 239,6 | 2,4 | 3,4 | <1 | 182 |

*T0 (NPK), T1 (potassium fertilization), T2 (fertilization with seaweed-based biostimulant), T3 (potassium nitrate based fertilization), T4 (potassium and algae-based biostimulant fertilization) and T5 (potassium fertilization and amino acid based biostimulant)*

## 4. CONCLUSIONS

We achieved an improvement in production by making different extra biostimulant contributions, which can be said to replace, at least under our working conditions, fertilizers of an inorganic origin. This means it is possible to maintain or even enhance yields in this type of growing given that we slightly increased production in our study. At the same time, we cultivated in a more environmentally friendly way, highlighting the extra supply of potassium and amino acid biostimulant among the applied treatments. On the other hand, none of the treatments altered the chemical composition nor the organoleptic quality of the oils, so the specific characteristics of the oils from the studied varieties were maintained in the implementation of the different fertilizing treatments.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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