

The use of biostimulants in high-density olive growing. Quality and production.

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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.

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).

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45 We observed an improvement with regard to the rest of treatments in the pomological
46 parameters of the olives when applying the potassium and amino acid biostimulant, while the
47 quality of the oils was not affected by the type of fertilization applied in each treatment.

48

49 **Introduction**

50

51 The olive tree is a traditional growing throughout the Mediterranean Basin and it plays a key
52 role in the so-called Mediterranean Diet (Lopez-Cortes et al, 2013). At present, the surface
53 cultivated in Spain of olive grove is 2.697.445 hectares, which supposes more than 50 % of
54 the surface devoted to the cultivation of woody species in this country. Its oil is said to have
55 nutraceutical properties, mainly due to its monounsaturated fatty acids, polyphenols and
56 tocopherols contents, which provide antioxidant, antimicrobial and carcinogenic activities,
57 among others. (Tekaya et al, 2014).

58

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

63

64 It is well documented that a suitable irrigation regime increases the size and weight of the
65 olives, in addition to improving the pulp/endocarp relation (Attalla et al, 2011), the difference
66 is greater when a custom fertilization is applied (Rosati et al, 2014). The use of fertilizers
67 exceeds 100 billion kilograms per year. This value has increased steadily in recent years
68 (Rubio-Covarrubias et al, 2008), along with the introduction of growings in high-density
69 systems, which increase fertilizer consumption and can lead to overuse contamination
70 (Neilsen et al, 1997) producing salinization and sodification of soils (Ramos et al, 2019).

71

72 In general, biostimulants have been described as products that contain substances and/or
73 microorganisms whose function is to stimulate natural processes, to enhance nutrient uptake,
74 and to improve nutrient use efficiency, tolerance to abiotic stress, and crop quality when
75 applied to plants or the rhizosphere. Council (EBIC, 2012). According to *Chen et al* (2001)
76 this type of compounds enhance soil microbial activity, thereby improving the fungal and
77 bacterial activity in the long term, as well as improving the crop itself.

78

79 Algae extracts are one of the most important components in the composition of biostimulants.
80 They enhance plant development and are beneficial for both human and animal health (Khan
81 et al, 2000). The major components of commercial SWE are polysaccharides, followed by
82 phenolics, vitamins precursors, osmolytes (mannitol), phytohormones, and hormone-like
83 compounds (Battacharyya et al, 2015). Furthermore, they improve plant resistance to both
84 biotic and abiotic stress (Nabti et al, 2016), activating different metabolites that provide the
85 plant with a better defense against pathogens (Farid et al, 2019).

86

87 The market for biostimulants has not stopped growing since the year 2013, at the rate of
88 about 12 % per year, and their main destinations are European holdings, which meant more
89 than 6 million hectares in our continent that year (Calvo et al, 2014).

90
91 In addition to improving plant development, biostimulants increase the biomass in different
92 crops such as the almond tree (Saa et al, 2015). Another type of products that falls within the
93 definition of biostimulants, such as compost, improves the development and growth of peach
94 trees (Baldi et al, 2014). On the other hand, it is important to point out that there is not only
95 an increase in the productions, but also an organoleptic improvement of production in the
96 case of fruit trees (Tanou et al, 2017).

97
98 Some studies consider that fertilization has no effect on the organoleptic characteristics of the
99 product obtained, however, it can alter the composition of compounds such as polyphenols in
100 olive oils (Tekaya et al, 2014).

101
102 It has also been written that products grown in more environment-friendly conditions are
103 tastier (Rosati et al, 2014), on the other hand, oils show a higher content of monounsaturated
104 fatty acids (Bourne and Prescott, 2002). People have been proven to have a greater interest in
105 pesticide-free products that present some type of certification, such as ecological or organic
106 products (Byrne et al., 1991), so it is interesting to carry out studies in this area.

107
108 Each cultivar possesses singular characteristics in composition of virgin olive oil (Vidal et al,
109 2019), but the location and climatic conditions also influence in this characteristics
110 (Fregapane and Salvador, 2018).

111
112 The Arbequina variety is known for adapting to high density cultivation, it is a Spanish origin
113 cultivar whose fruits are small and round and its oils are smooth only slightly bitter and
114 peppery.

115
116 The Koroneiki is a Greek origin variety, it is very important in the production of oils. It
117 provides an intense green color which is very much appreciated by consumers. Its fruits are
118 large and oval, the oils obtained from its olives have a bitter and peppery taste, as opposed to
119 the Arbequina cultivar.

120
121 Despite all the benefits involved in the use of this type of products (biostimulant fertilizing
122 treatments), it is necessary to understand that carrying out a fertilization process of this type
123 is a complex activity that requires meeting the nutritional needs of the plant as well as
124 ensuring soil fertility (Ibrahimi and Gaddas, 2015). That is why this study aims to evaluate
125 the possible production and quality differences in an intensive cultivation of olive trees by
126 comparing biostimulant fertilizing treatments in order to prove if it is possible to maintain the
127 productive performance of an intensive system holding, using environmentally friendly
128 fertilization. Our study focuses on the search for an environmentally friendly fertilization as
129 well as the achievement of an optimum production while maintaining the highest standards of
130 both chemical and organoleptic qualities.

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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 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

175 was then poured into a blender in a bath to keep the temperature below 21 °C and thus extract
176 the individual oil in each of the fertigation trials. After this process was completed, the mass
177 was then centrifuged to separate the oil from the solid and aqueous phase obtained after the
178 blending phase.

179

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

187

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

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

210 α -Tocopherol was evaluated following AOCS method Ce 8–89. A solution of oil in hexane
211 was analyzed on an Agilent Technologies HPLC system (1100 series) on a silica gel
212 Lichrosorb Si-60 column (particle size 5 μ m \times 250 mm \times 4 mm i.d. of Sugerlabor, Madrid,
213 Spain) using n-hexane/2-propanol (98.5/1.5, vol/vol) at a flow rate of 1 mL/min. A
214 fluorescence detector (Thermo-Finnigan FL3000) was used, with excitation and emission
215 wavelengths set at 290 and 330 nm, respectively.

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

220

221 **Results and Discussion**

222

223 In the pomometric characterization of the Arbequina variety, we observed differences
224 between the studied treatments in the size and weight of the fruits and their endocarps, as
225 reflected in Table 2. In the two studied campaigns, we could observe that the heaviest fruits
226 were the ones who had received an extra intake of potassium and amino acids biostimulant
227 (T5), with an average weight between 1.30 and 1.38 grams in each campaign, while the
228 lighter fruits were the control treatment with a weight between 0.92 and 0.93 grams in each
229 campaign, this has an impact on the pulp endocarp that usually marks the performance of the
230 fruits, so it is one of the most relevant values that are generally studied. Thus, the treatments
231 that represented the maximum and minimum values for this parameter were repeated, and the
232 fruits with a higher pulp/endocarp relation, ranging between 76 % and 78 %, came from trees
233 treated with an extra supply of amino acids and potassium (T5), while the fruits of the control
234 treatment that received conventional NPK fertilization, recorded a lower pulp/endocarp
235 relation of between 65 % and 70 %, just like Laila et al (2013), in our study, we improved the
236 caliber of the olives with biofertilizer contributions.

237

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

244

245 On the other hand, the treatment with lighter fruits and less pulp/endocarp relation was the
246 control treatment. Chouliaras et al (2009) obtained an improvement in the pomometry of the
247 fruits of this variety when applying algae extract biostimulants, similar to our T2 treatment,
248 while those who had lower values for the pomometric parameters in study were those in the
249 control treatment, with a fruit weight between 0.45 and 0.54 grams, which is reflected in
250 Table 3, where the pulp/endocarp relation of the fruits under the T5 treatment (extra supply of
251 potassium and amino acid based biostimulant) presented an average value in both campaigns
252 of 73 %, whereas in the control treatment, they varied between 61 % and 64 %.

253

254 With regard to the productions per tree, the same applies for the pomometry, trees that
255 showed a better performance, and therefore increased production during the two campaigns
256 of cultivation under study, were those belonging to the crop lines treated with an extra supply
257 of potassium and amino acid biostimulant (T5) for both varieties. There was an average
258 production of 6.35 kg per tree in the trees of the Arberquina variety in which this treatment
259 was applied, while the trees in the control treatment barely achieved an average production of
260 4.87 kg per tree. On the other hand, in the Koroneiki variety, production was 7.45 kg per tree

261 in the lines treated with an extra supply of potassium and amino acid biostimulant (T5), while
262 the trees of the control treatment lines obtained an average production of 4.8 kg per tree.

263

264 After analyzing the composition of the obtained oils, as shown in tables 4 and 5, we found
265 that the fatty acids, polyphenols and tocopherols contents were not significantly affected in
266 any of the various combinations variety-treatment, there were only small variations in some
267 of them. However, other authors such as Tekaya et al (2014) have seen significant variations
268 in the content of tocopherols, while Fernández-Escobar et al (2006) observed variations in the
269 polyphenol content. This may be due to the fact that our study was conducted in a high
270 density growing which was not the case in the studies of these authors.

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

279

280 **Conclusions**

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

291

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293

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400

401 **Tables**

402 Table 1. Composition of applied treatments^o

Treatment	Composition
T0	NPK-based fertilization (130 UF N, 35 UF P ₂ O ₅ , 180 UF K ₂ O)

T1	Potassium fertilization (60 % K ₂ O)
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 ₂ O)
T4	Potassium and algae-based biostimulant fertilization (60 % K ₂ O) + (2,08 % Bo, 0,02 % Mo and GA142 seaweed filtrate)
T5	Potassium fertilization and amino acid based biostimulant (60 % K ₂ O) + (12 % Aminoácidos libres + 8,5 % N + 2,5 % MgO)

404 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 ^c	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

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

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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 ^c	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

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

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

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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

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

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422 **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

423 T0 (NPK), T1 (potassium fertilization), T2 (fertilization with seaweed-based biostimulant), T3 (potassium nitrate based fertilization), T4

424 (potassium and algae-based biostimulant fertilization) and T5 (potassium fertilization and amino acid based biostimulant).

