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

9	
10	
11	Abstract
12	
13	Due to the increase of high-density holdings, especially of olive trees, the nutritional
14	requirements of the plants are higher per unit area, which implies that a greater contribution of
15	fertilizers to the soil is needed. Opting for fertilizers of inorganic origin will produce an increase
16	in the pollution of the soil.
17	
18	In the face of this possible soil contamination, our aim is to analyze the effect of biostimulants
19	as an alternative to chemical fertilizers, to steadily produce and maintain high quality standards
20	during the life of the crop. Our objective is using more environmentally friendly products in
21	order to satisfy one of the most important demands from both consumers and the authorities.
22	
23	In this study, we carried out five different treatments in addition to a control treatment with a
24 25	supply of NPK, from inorganic products, which are used to control fertilization with a solution
25 26	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
20 27	Koroneiki.
28	
29	This study was developed in the farm <i>Pozohondo</i> , which is located in a crop zone by the
30	<i>Palancia</i> river (Castellón, Valencia, Spain), in the southeast of the Iberian Peninsula, where
31	the olive trees were established in a high-density system with a planting framework of 4×1.5
32	m. We ensured an exhaustive control of the nutritional needs of the holding by using a
33	fertigation system.
34	
35	We could notice differences in the productions of each applied treatment, avoiding any possible
36	biases through the additional control of 100 randomly selected olives from each of the samples.
37	We analyzed the quality of the olive oil obtained from the production of each treatment by
38	measuring the fatty acids, tocopherols and polyphenols contents. We also carried out an
39	organoleptic tasting analysis following the rules of the International Olive Committee (IOC).
40	
41	

42 We observed an improvement with regard to the rest of treatments in the pomological 43 parameters of the olives when applying the potassium and amino acid biostimulant, while the

44 quality of the oils was not affected by the type of fertilization applied in each treatment.

45

46 Introduction

47

48 The olive tree is a traditional growing throughout the Mediterranean Basin and it plays a key

role in the so-called Mediterranean Diet (Lopez-Cortes et al, 2013). Its oil is said to have

50 nutraceutical properties, mainly due to its monounsaturated fatty acids, polyphenols and

51 tocopherols contents, which provide antioxidant, antimicrobial and carcinogenic activities,

- 52 among others. (Tekaya et al, 2014).
- 53

54 There is a clear tendency nowadays towards the use of environmentally friendly cropping 55 techniques, there is a special interest in the practice of organic fertilization with products 56 coming from extracts of algae and/or crops, which provide a high organic matter content that 57 delivers the necessary nutrients to the plant.

58

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 (Attalla et al, 2011), the difference is greater when a custom fertilization is applied (Rosati et al, 2015). The use of fertilizers exceeds 100 billion kilograms per year. This value has increased steadily in recent years (Rubio-Covarrubias et al, 2008), along with the introduction of growings in high-density systems, which increase fertilizer consumption and can lead to overuse contamination (Neilsen and Neilsen, 1997).

66

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 (EBIC, 2012). According to *Chen et al* (2001) 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.

73

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 (*Khan et al*, 2000). Furthermore, they improve plant resistance to both biotic and abiotic stress (*Nabti et al*, 2016).

78

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 (Calvo et al, 2014).

82

In addition to improving plant development, biostimulants increase the biomass in different
crops such as the almond tree (Saa et al, 2015). Another type of products that falls within the
definition of biostimulants, such as compost, improves the development and growth of peach

trees (Baldi et al, 2014). 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

88 fruit trees (Tanou et al, 2017).

89

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 (Tekaya et al, 2014).

93

94 It has also been written that products grown in more environment-friendly conditions are tastier 95 (Rosati et al, 2014), on the other hand, oils show a higher content of monounsaturated fatty 96 acids (Bourne and Prescott, 2002). People have been proven to have a greater interest in 97 pesticide-free products that present some type of certification, such as ecological or organic 98 products (Byrne et al., 1992), so it is interesting to carry out studies in this area.

99

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.

103

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.

108

109 Despite all the benefits involved in the use of this type of products (biostimulant fertilizing 110 treatments), it is necessary to understand that carrying out a fertilization process of this type is 111 a complex activity that requires meeting the nutritional needs of the plant as well as ensuring 112 soil fertility (Ibrahimi and Gaddas, 2015). That is why this study aims to evaluate the possible 113 production and quality differences in an intensive cultivation of olive trees by comparing 114 biostimulant fertilizing treatments in order to prove if it is possible to maintain the productive 115 performance of an intensive system holding, using environmentally friendly fertilization. Our 116 study focuses on the search for an environmentally friendly fertilization as well as the 117 achievement of an optimum production while maintaining the highest standards of both 118 chemical and organoleptic qualities.

119

120 Materials and Methods

121

122 The study was carried out in an olive tree exploitation located in the province of Castellón 123 (Spain) (39°53'50.1"N 0°31'28.0"W) in the southeast of the Iberian Peninsula, in an area with 124 an average temperature of 14.2°C and an average annual rainfall of 384 mm per year. The 125 planting pattern was 1.50 x 4.00 meters, for trees of two of the main varieties that are used in 126 this type of growings, the Arbequina and the Koroneiki varietyies, that are 2.50 meters high, 127 20 years of age, and in full production. The plot had a fertigation system with which the 128 contributions of irrigation and fertilization were made, irrigation was 3500 m3 per hectare per 129 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).
- 133

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.

137

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).

145

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.

150

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.

155

156 Once the pomological analysis was carried out, we conducted a pomometric analysis of the 157 olives by measuring the weight, length, width A and width B of each of them, after which we 158 proceeded to the study of the endocarps, and at the same time obtained the pulp/endocarp 159 relation.

160

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.

167

168 Once the oils were separated, a sample of each of them was taken to be analyzed in the 169 laboratory, in order to get the parameters that indicate their quality from a chemical point of 170 view by analyzing the polyphenols, tocopherols and fatty acids contents. This process was 171 aimed at verifying that they were extra virgin olive oils (EVOO), complying with the highest 172 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 rulesof the International Olive Oil Council (IOOC).

175

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–

- 181 lignoceric acid (C24:0).
- 182

183 Three sterols were examined: β -sitosterol, stigmasterol and campesterol. The oil sample was saponified with an ethanolic potassium hydroxide solution. The unsaponifiable fraction was 184 185 removed with an ethyl ether. The unsaponifiable sterol fraction was separated by silica gel plate 186 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 187 chromatograph, equipped with an FID-300, which worked at 290 °C. The sample was injected 188 189 at 280 °C, following an isothermal process at 265 °C for 45 min using a HP-5MS capillary 190 column (30 m \times 0.25 mm \times 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 191 temperature was 300 °C; and the detector temperature was 290 °C. The injection volume was 192 193 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 194 195 internal pattern (5- α -cholestanol). The sterol concentration was expressed as mg/100 g of fatty 196 matter. The area of peaks generated by the sterols was carried out by an automatic integrator.

197 α -Tocopherol was evaluated following AOCS method Ce 8–89. A solution of oil in hexane 198 was analyzed on an Agilent Technologies HPLC system (1100 series) on a silica gel Lichrosorb 199 Si-60 column (particle size 5 μ m × 250 mm × 4 mm i.d. of Sugerlabor, Madrid, Spain) using 200 n-hexane/2-propanol (98.5/1.5, vol/vol) at a flow rate of 1 mL/min. A fluorescence detector 201 (Thermo-Finnigan FL3000) was used, with excitation and emission wavelengths set at 290 and 202 330 nm, respectively.

203

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, distinguishing between treatments of the same variety, PCAs (Principal Components Analysis) to differentiate the general behavior of each of the varieties to the effect of the applied treatments.

209

211

210 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

- 215 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

217 the control treatment with a weight between 0.92 and 0.93 grams in each campaign, this has an 218 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 219 maximum and minimum values for this parameter were repeated, and the fruits with a higher 220 221 pulp/endocarp relation, ranging between 76 % and 78 %, came from trees treated with an extra 222 supply of amino acids and potassium (T5), while the fruits of the control treatment that received 223 conventional NPK fertilization, recorded a lower pulp/endocarp relation of between 65 % and 224 70 %, just like Laila et al (2013), in our study, we improved the caliber of the olives with 225 biofertilizer contributions.

226

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.

233 On the other hand, the treatment with lighter fruits and less pulp/endocarp relation was the 234 control treatment. Chouliaras et al (2009) obtained an improvement in the pomometry of the 235 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 236 237 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 238 239 potassium and amino acid based biostimulant) presented an average value in both campaigns 240 of 73 %, whereas in the control treatment, they varied between 61 % and 64 %.

241

242 With regard to the productions per tree, the same applies for the pomometry, trees that showed 243 a better performance, and therefore increased production during the two campaigns of 244 cultivation under study, were those belonging to the crop lines treated with an extra supply of 245 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 246 247 applied, while the trees in the control treatment barely achieved an average production of 4.87 248 kg per tree. On the other hand, in the Koroneiki variety, production was 7.45 kg per tree in the 249 lines treated with an extra supply of potassium and amino acid biostimulant (T5), while the 250 trees of the control treatment lines obtained an average production of 4.8 kg per tree.

251

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 (2014) have seen significant variations in the content of tocopherols, while Fernández-Escobar et al (2006) observed variations in the polyphenol content. 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 varietytreatment 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.

267

268 Conclusions

269

270 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 271 an inorganic origin. This means it is possible to maintain or even enhance yields in this type of 272 growing given that we slightly increased production in our study. At the same time, we 273 274 cultivated in a more environmentally friendly way, highlighting the extra supply of potassium 275 and amino acid biostimulant among the applied treatments. On the other hand, none of the 276 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 277 278 implementation of the different fertilizing treatments.

285	
286	References
287 288 289 290 291	Attalla A.M., Abdel-Sattar M., Mahrous A.E. & Abdel-Azeez, A.A. (2011). Olive Trees Productivity in Response to Supplemental Irrigation under North-Western Coastal Conditions in Egypt. American-Eurasian J. Agric. & Envir. Sci. 11(5): 609-615.
292 293 294 295	Baldi E., Marcolini G., Quartieri M., Sorrenti G. & Toselli M. (2014). Effects of organic fertilization on nutrient concentration and accumulation in nectarine (<i>Prunus pérsica</i> var. Nucipersica) tres: The effect of rate of application. Scientia Horticulturae, 179: 174-179.
296 297 298 299 300	Borges T.H., Pereira J.A., Cabrera-Vique C., Lara L., Oliveira A.F. & Seiquer I. (2017). Characterization of Arbequina virgin olive oils produced in different regions of Brazil and Spain: Physicochemical properties, oxidative stability and fatty acid profile. Food Chemistry, 215: 454-462.
301 302 303	Calvo, P., Nelson, L. & Kloepper J. W. (2014). Agricultural uses of plant biostimulants. Plant soil, 383: 3-41.
304 305 306 307 308	Boussadia O., Steppe K., Zgallai H., Ben el Hadj S., Braham M., Lemeur R. & Van Labeke M.C. (2010). Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars 'Meski' and 'Koroneiki'. Scientia Horticulturae. 123: 336- 342.

316

319

326

330

- Chen S., Subler S. & Edwards C. A. (2002). Effects of agricultural biostimulants on soil microbial
 activity and nitrogen dynamics. Applied Soil Ecology. 19, 249-259.
- Chouliaras V., Tasioula M., Chatzissavvidis C., Therios I. & Tsabolatidou E. (2009). The effects of a
 seaweed extract in addition to nitrogen and boron fertilization on productivity, fruit
 maturation, leaf nutritional status and oil quality of the olive (Olea europea L.) cultivar
 Koroneiki. Journal of Sci. Food Agric. 89: 984-988.
- COI. Método de valoración organoléptica del aceite de oliva virgen extra que opta a una denominación de origen. COI/T.20/Doc nº 22. Noviembre 2015.
- Du Jardin, P. 2015. Plant biostimulants: Definition, concept, main categories and regulation.
 Scientia Horticulturae, 196, 3-14.
- Fernandez-Escobar, R., Beltran, G., Sanchez-Zamora, M. A., Garcia-Novelo, J.,
 Aguilera, M. P., & Uceda, M. (2006). Olive oil quality decreases with nitrogen over fertilization.
 HortScience, 41, 215–219.
- Fernández-Escobar R., Marin L., Sánchez-Zamora M.A., García-Novelo J.M., Molina-Soria C. & Parra
 M.A. (2009). Long-term effects of N fertilization on cropping and growth of olive trees and on
 N accumulation in soil profile. European Journal of Agronomy. 31: 223-232.
- Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P., Hodges, D. M., Critchley, A.
 T., Craigie, J. S., Norrie, J. & Prithiviraj B. (2009). Seaweed Extracts as Biostimulants of Plant
 Growth and Development. J. Plant Growth Regul. 28: 386-399.
- Laila, F. H., Shahin, M. F., Merwad, M. A., Khalil, F.H. & El-Hady, E. S. (2013). Improving fruit quality
 and quantity of "Aggizi" olive trees by application of humic acid during full Bloom and fruit
 set stages. Middle East j, 2(2), 44-50.
- López-Cortés, I., Salazar-García, D.C., Velázquez-Martí, B. & Salazar D.M. (2013) Chemical
 characterization of traditional varietal olive oils in East of Spain. Food Research
 International. 54: 1934-1940
 342
- Nabti, E., Jha, B. & Hartmann, A. (2017). Impact of Seaweeds on Agricultural Crop Production as
 Biofertilizer. Int. J. Environ. Sci. Technol. 14: 1119-1134.
- Neilsen D., Millard P., Neilsen G.H. & Hogue E.J. (1997). Sources of N for leaf growth in a highdensity appel (*Malus domestica*) orchard irrigated with ammonium nitrate solution. Tree
 Physiology. 17(11): 733-739.
- Saa, S., Olivos-Del Rio, A., Castro, S. & Brown, P. H. (2015). Foliar application of microbial and plant
 based biostimulants increases growth and potassium uptake in almond (*Prunus dulcis* [Mill.]
 D. A. Webb). Frontiers in plant science, (6), 87.
- Tanou, G., Ziogas, V. & Molassiotis, A. (2017). Foliar nutrition, Biostimulants and Prime-Like
 Dynamics in Fruit Tree Physiology: New Insights on an Old Topic. Frontiers in plant science,
 8, 75.
- Tekaya M., Mechri B., Bchir A., Attia F., Cheheb H., Daassa M. & Hammami M. (2013). Effect of nutrient-based fertilisers of olive trees on olive oil quality. Journal of Sci. Food Agric. 93: 2045-2052.
- 361

349

Tekaya M., Mechri B., Bchir A., Cheheb H., Attia F., Chraief I., Ayachi M., Boujneh D. & Hammami M.
(2014). Changes in the profiles of mineral elements, phenols, tocopherols and soluble
carbohydrates of olive fruit following foliar nutrient fertilization. LWT-Food Science and
Technology. 59: 1047-1053.

366

367 Tables

368 Table 1. Composition of applied treatments^o

Treatment	Composition
TO	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)
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)
T5	Potassium fertilization and amino acid based biostimulant (60 % K ₂ O) + (12 % Aminoácidos libres + 8,5 % N + 2,5 % MgO)

		Cult	tivar Arbequina first	year		
	TO	T1	T2	T3	T4	T5
Fruit weight (g)	$0.93\pm0.19^{\text{e}}$	1.14 ± 0.22^{c}	$0.99\pm0.29^{\rm d}$	$1.03\pm0.27^{\text{d}}$	1.21 ± 0.23^{b}	1.38 ± 0.24^{a}
Fruit length (mm)	12.41 ± 1.14^{d}	13.49 ± 1.12^{b}	$12.66 \pm 1.25^{\circ}$	13.36 ± 1.16^{b}	13.46 ± 1.02^{b}	14.56 ± 1.11^{a}
Fruit width A (mm)	$10.60\pm0.93^{\text{d}}$	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}	$10.77 \pm 1.02^{\circ}$	11.09 ± 0.86^{b}	12.39 ± 0.87^{a}
Endocarp weight (g)	0.27 ± 0.06^{d}	$0.30\pm0.06^{\text{b}}$	0.29 ± 0.07^{cd}	0.30 ± 0.06^{b}	$0.33\pm0.06^{\text{a}}$	0.30 ± 0.05^{bc}
Endocarp length (mm)	9.33 ± 0.81^{d}	10.05 ± 0.85^{b}	$9.76\pm0.94^{\rm c}$	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}	$6.82\pm0.42^{\text{a}}$	6.52 ± 0.36^{cd}
Pulp/endocarp relation	$0.70\pm0.04^{\rm c}$	$0.74\pm0.04^{\text{b}}$	$0.70\pm0.05^{\rm c}$	$0.70\pm0.04^{\rm c}$	0.73 ± 0.03^{b}	$0.78\pm0.03^{\text{a}}$
		Culti	var Arbequina secono	l year		
	TO	T1	T2	T3	T4	Т5
Fruit weight (g)	$0.92\pm0.21^{\text{d}}$	0.96 ± 0.32^{d}	1.06 ± 0.20^{b}	1.00 ± 0.21^{c}	1.03 ± 0.23^{bc}	$1.30\pm0.30^{\rm a}$
Fruit length (mm)	12.93 ± 0.97^{d}	12.50 ± 1.30^{d}	13.03 ± 1.13^{b}	13.41 ± 1.27^{bc}	$12.89 \pm 1.18^{\circ}$	13.91 ± 1.15^{a}
Fruit width A (mm)	10.44 ± 0.86^{c}	$10.32 \pm 1.18^{\text{d}}$	$11.12\pm0.85^{\rm c}$	10.96 ± 0.83^{b}	$10.84 \pm 0.97^{\circ}$	12.01 ± 1.06^{a}
Fruit width B (mm)	10.11 ± 0.82^{d}	$10.00 \pm 1.15^{\rm d}$	10.72 ± 0.82^{b}	10.79 ± 0.91^{b}	$10.49 \pm 0.92^{\circ}$	11.75 ± 1.05^{a}
Endocarp weight (g)	0.31 ± 0.06^{b}	$0.28\pm0.06^{\rm c}$	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}	$9.92\pm0.92^{\rm c}$	10.49 ± 1.09^{a}	10.17 ± 1.04^{b}	$10.19\pm0.86^{\text{b}}$	10.16 ± 0.88^{b}

Table 2. Fruit pomometric characterization of cultivar Arbequina

Endocarp width A (mm)	6.87 ± 0.50^{bc}	$6.72\pm0.51^{\text{e}}$	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}

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

372 biostimulant fertilization) and T5 (potassium fertilization and amino acid based biostimulant).

373 Different letters indicate statistical significant differences in a 95 %.

374

375 Table 3. Fruit pomometric characterization of cultivar Koroneiki

	Cultivar Koroneiki first year												
	TO	T1	T2	T3	T4	T5							
Fruit weight (g)	$0.54\pm0.18^{\text{d}}$	$0.73\pm0.13^{\text{b}}$	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}	$14.79 \pm 1.11^{\text{b}}$	14.01 ± 1.64^{c}	15.04 ± 1.21^{ab}	15.12 ± 1.35^{a}	15.19 ± 1.44^{a}							
Fruit width A (mm)	$8.11\pm0.89^{\text{e}}$	9.01 ± 0.65^{c}	$8.85 \pm 0.83^{\text{d}}$	$9.27\pm0.72^{\rm b}$	$9.49\pm0.73^{\rm a}$	9.52 ± 0.89^{a}							
Fruit width B (mm)	7.81 ± 0.95^{d}	8.65 ± 0.64^{bc}	$8.59\pm0.81^{\text{c}}$	8.78 ± 0.73^{b}	$9.19\pm0.75^{\text{a}}$	9.13 ± 0.87^{a}							
Endocarp weight (g)	0.18 ± 0.04^{c}	0.21 ± 0.04^{b}	$0.19\pm0.05^{\rm c}$	$0.19\pm0.04^{\rm c}$	0.22 ± 0.05^{a}	0.21 ± 0.04^{b}							
Endocarp length (mm)	$10.98 \pm 1.05^{\rm c}$	$11.66\pm0.91^{\texttt{a}}$	$11.07 \pm 1.24^{\rm c}$	11.37 ± 0.87^{b}	11.82 ± 1.06^{a}	11.75 ± 1.09^{a}							
Endocarp width A (mm)	$5.40\pm0.36^{\rm c}$	5.62 ± 0.38^a	5.44 ± 0.38^{bc}	$5.47\pm0.32^{\text{b}}$	$5.58\pm0.42^{\rm a}$	$5.58\pm0.35^{\rm a}$							
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}	$0.71\pm0.04^{\rm c}$	0.72 ± 0.04^{bc}	0.73 ± 0.03^{ab}	$0.72\pm0.04^{\rm c}$	$0.73\pm0.05^{\rm a}$							
Cultivar Koroneiki second year													
	Control	T1	Τ2	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}	$14.43 \pm 1.39^{\text{b}}$	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}	$8.00\pm0.42^{\rm c}$	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}	$0.18\pm0.03^{\text{c}}$	$0.18\pm0.03^{\text{c}}$	0.20 ± 0.04^{a}	0.20 ± 0.04^{b}
Endocarp length (mm)	$10.69\pm0.76^{\rm c}$	$10.59\pm0.75^{\rm c}$	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}	$5.30\pm0.27^{\text{c}}$	$5.32\pm0.32^{\rm c}$	$5.49\pm0.32^{\rm a}$	5.37 ± 0.36^{b}
Endocarp width B (mm)	5.11 ± 0.24^{e}	$5.12\pm0.28^{\text{de}}$	$5.17\pm0.27^{\text{c}}$	$5.16\pm0.30^{\text{cd}}$	5.37 ± 0.31^{a}	5.25 ± 0.36^{b}
Pulp/endocarp relation	$0.61\pm0.08^{\text{e}}$	0.69 ± 0.06^{c}	0.66 ± 0.07^{d}	0.72 ± 0.03^{b}	0.69 ± 0.06^{c}	$0.73\pm0.05^{\rm a}$

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

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

378 Different letters indicate statistical significant differences in a 95 %.

379

380 Tabla 4. Olive oils fatty acids composition of the studied cultivars

Cultivor	Treatmen						Fatty aci	ds comp	oosition					
Cultivar	t	Miristic	Palmitic	Palmitoleic	Margaric	Margaroleic	Estearic	Oleic	Linoleic	Linollenic	Araquidic	Gadoleic	Behenic	Lignoceric
Arbequina	Т0	<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	Т0	<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

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

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

384 Different letters indicate statistical significant differences in a 95 %.

385

386

387

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

Cultivar	Treatment	Isomers	s Trans		Tocopherols/Tocotrienols							
	Treatment	Trans Oleics	Tr L+Tr Ln	Total Tocopherols	a-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			

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

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

391 Different letters indicate statistical significant differences in a 95 %.