Use of digestate as an alternative to chemical fertilizer: effects on growth and crop quality

Digestate benefits for crop quality

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ABSTRACT

Nowadays, the recycling of organic wastes represents a key factor for improving soil and crop quality with socio-economic and environmental benefits. In recent years, the production of digestate, residue of biogas plants, significantly increased with a crescent need to be sustainable disposal. The use of digestate as soil amendment has been widely studied but few researches evaluated the digestate effects on crop quality. Since it has been proven that digestate, increased soil fertility, we hypothesized that it could result in the same way of organic fertilizers increasing crop quality. To verify this hypothesis, we analyzed the effects of two digestates on *Cucumber* quality. Cucumber was chosen because it is an important old crop used worldwide as fresh food, and in cosmetic and pharmaceutic industries. Results showed that digestates increased its content of phenols and flavonoids with antioxidant activity. Nehoesperidine and hesperitin with well-known antioxidant, anti-inflammatory and anti-carcinogenic properties as well as naringin and narirutin with the power of reducing the risk of atherosclerosis and cardiovascular complications manifested only in amended cucumbers. These results highlighted the potentiality of digestates as health-promoting bio compounds conferring on it a new feature in the smart-sustainable agriculture as inducer of phytochemicals to produce functional food.

Keywords: anaerobic digestate; *Cucumis sativus;* flavonoids; phenols; phytochemicals

Introduction

Nowadays, the recycling of organic wastes in agriculture represents the most promising alternative to mitigate environmental problems improving soil productivity and crop quality with socio-economic benefits. In this regard digestate, the residue of biogas plant, a fully fermented nutrient-rich material, could be used as alternative to synthetic fertilizer (Eickenscheidt et al., 2014; Koszel and Lorencowicz, 2015; Vázquez-Rowe et al., 2015) as such or separated into liquid and solid fractions (Mata-Alvarez et al., 2014; Pivato et al., 2016). The solid fraction is high in dry matter (DM), N and P, conversely the liquid fraction, is low in DM and equally rich in nutrients so that can be considered equivalent to liquid commercial fertilizers. Several publications have demonstrated the effectiveness of digestate as suitable

nutrient source in agriculture (Chantigny et al., 2008; Arthurson, 2009; Panuccio et al., 2016; Muscolo, et al., 2017). Moller and Muller (2012) stated an high "degree of freedom" of total amount of mobile organic fertilizers when digestate was used in soil, with an increase in soil N and N use efficiency, in comparison to the direct soil incorporation of green manures or crop post-harvest residues. Furukawa and Hasegawa (2006) demonstrated, on spinach and komatsuna production, that biogas residue from source-separated household wastes was comparable to NPK fertilizers. Subsequently, Panuccio et al (2016) showed that the chemical composition and quality of digestate in terms of organic matter and nutrient availability drove the agronomic properties of digestate and its impact on soil properties and plant nutrition. Numerous recent investigations highlighted that organic amendments have an influence on the nutritional quality of crops (Dumas et al., 2003), estimating that organic vegetables may contain 10-50% higher defense-related secondary metabolites than conventionally grown vegetables (Olsson et al., 2006; Mitchell et al., 2007; Sousa et al., 2008). In this regard, a comparative study of organic and conventionally grown grapes showed that organic amendment increased phenolics in the skin compared with conventional fertilization (Malusà et al., 2004). Subsequently, Selma et al. (2010) demonstrated that organic soil amendments with sewage sludge and urban solid wastes increased glucosinolates as well as flavonols and anthocyanins in rocket leaves (Eruca sativa) (Arthurson, 2009). Based on the above considerations, anaerobic digestates could be used for a smart agriculture to increase the production of bioactive compounds in crops for producing functional food. A significant part of the plant bioactive compounds is the results of secondary metabolism activated at different extent by external factors such as, abiotic and biotic stress, amendment and mineral nutrition. The aim of present study was to estimate the effects of two different digestates, separated in solid and liquid fractions, previously characterized and tested on soil quality (Panuccio et al., 2016; Muscolo et al., 2017) on secondary metabolite (phytonutrients) production in cucumber. Cucumber (*Cucumis sativus*) is one of the oldest cultivated crops, recommended in the diet for its very low-calories, saturated fat and cholesterol content, high amount of potassium, vitamin-C, vitamin-A and vitamin-K (Mukherjee et al., 2013). *Cucumis sativus* contains also a large variety of biologically active, non-nutritive compounds known as phytochemicals, such as alkaloids, flavonoids, steroids, tannins among others (Rajasree et al., 2016) that make it really attractive from cosmetic and pharmaceutical industries. In recent years, a great number of epidemiological studies evidenced the potentiality of fruits and vegetables as health-promoting foods for their richness in numerous phytochemicals, including antioxidants (Della Penna, 1999), with beneficial effects on human health offering protection against cardiovascular disease, development of cancer,

diabetes and osteoporosis (Babajide et al., 2013; Mukherjee et al., 2013). It has been well demonstrated that the daily consumption of plant-based foods, plays important roles in the prevention of chronic and degenerative diseases including decrease in mortality risk and lower incidence of cardiovascular disease (Arai et al., 2000; Hu, 2003; Trichopoulou et al., 2014). Our specific aim was to confer to digestate a new feature in the smart-sustainable agriculture to produce functional food with nutritional and health-beneficial values.

Materials and Methods

Plant material and experimental conditions

The experiment was conducted in controlled environment for 3 months, in greenhouse at a temperature of 23 ± 2 °C. The experiment was replicated for three consecutive years. Plastic pots (26 cm diameter × 27 cm height) were filled with 4,5 Kg of an alkaline sandy-loam soil, and two digestates, previously characterized and tested as organic fertilizer (Panuccio et al., 2016; Muscolo et al., 2017), were added. The two digestates were arbitrarily called Fattoria and Uliva, on the basis of the feedstock used to fed the two digesters (Panuccio et al., 2016). In this experiment the digestates were separated in liquid and solid fractions. The solid fractions Fattoria (SF) and Uliva (SU) were used at the concentration of 25, 50 and 75% and the liquid fractions Fattoria (LF) and Uliva (LU) at the concentration of 10, 20 and 30%. Unfertilized soil was used as control. The experiment comprised three replicates, pots were arranged randomly on benches. During the experiment, the soil moisture was maintained at 70% of the field capacity in all treatments. 3 months after treatments, plant height, leaf number and leaf area, collar diameter, flower and fruit numbers were evaluated.

Extraction of antioxidant and flavonoid compounds

Frozen sample (5g) was homogenized with 15 mL of MeOH:H_2O (80:20), centrifuged at 3000 rpm for 15 minutes and filtered through a 0.45 µm filter (Millipore Corporation, Bedford, USA). The cucumber extract was frozen at -80°C until analysis.

Total phenol and total flavonoid assay

Total phenol content was detected with the Folin–Ciocalteu reagent with a modified procedure described by Singleton et al. (1999). 500 µL of the aqueous extract was mixed with 250 µL of Folin-Ciocalteu reagent and 2 mL of a 20% Na_2CO_3 aqueous solution, the mixture was filled up to 50 mL with deionised

water and placed in the dark for 1 h. Afterward the absorbance was measured at 765 nm using a UV–Vis Agilent 8453 spectrophotometer (Agilent Technologies, CA USA). The results were expressed as mg/L of gallic acid equivalents.

Total flavonoid content was determined following the colorimetric method of Yoo et al. (2004) modified as follows. 2 mL of aqueous extract was mixed with 300 µL of a 5 % $NaNO_2$ water solution. After 5 min, 600 µL of 10% $AlCl_3$ solution in water was added. After 5 min, 2 mL of 1 M NaOH was added to the mixture which was filled up to 10 mL with deionised water. The absorbance was measured at 510 nm using a UV–Vis Agilent 8453 spectrophotometer (Agilent Technologies, CA USA). The results were expressed as rutin equivalents (mg/L) by a calibration curve.

DPPH· and vitamin C assays

The antioxidant activity was detected with the DPPH· free radical assay (Brand-Williams et al., 1995). A 2.4 mL of 0.06 mM DPPH· methanolic solution was added to 100 µL of aqueous extract. The absorbance was measured at 515 nm. The DPPH· solution without the extract was used as a blank. The results were expressed according to the following equation:

% Inhibition = $((A_{t0}-A_{t5})/ A_{t0}) \times 100$; where A_{t0} is the DPPH· absorbance of blank (reference solution) and A_{t5} is the DPPH· absorbance of the sample.

Vitamin C was assayed according to the method of Kampfenkel et al. (1995) modified as follows. Sample (2 g) was homogenized in 8 mL 6% (w/v) trichloroacetic acid, centrifuged at 3500 rpm for 20 min and the supernatant was analysed. The assay is based on the reduction of Fe^{3+} to Fe^{2+} by vitamin C in an acidic solution. Fe^{2+} forms complexes with 2,20-bipirydyl, giving a pink colour with the maximum absorbance at 525 nm. The measurement was done in a UV–Vis Agilent 8453 spectrophotometer (Agilent Technologies, CA, USA). A calibration curve of ascorbic acid was prepared and the results were expressed as were expressed as mg of ascorbic acid /100 g fresh weight of cucumber.

Detection of individual phenolic and flavonoid compounds by HPLC analysis

Cucumber pulp (2.5 g) was homogenized with a 5 mL of MeOH:H_2O (80:20) solution, the mixture was centrifuged at 3000 rpm for 15 min and the supernatant was passed through a 0.45 µm filter (Millipore Corporation, Bedford, USA). The obtained cucumber extract was analysed immediately. Separation of phenolic and flavonoid compounds was performed by a HPLC/DAD Knauer system (Asi Advanced Scientific Instruments, Berlin, Germany), equipped with two pumps Smartiline Pump 1000, a Rheodyne

injection valve (20 µL) and a photodiode array detector UV/VIS provided with a semi micro-cell. UV spectra was recorded in the range of 210–365 nm and simultaneous detection by diode array was performed at 254, 280 and 365 nm. Processing data were carried out using Clarity Software (Chromatography Station for windows). Phenolic compounds were separated on a Phenomenex C18 column (250 mm x 4.6 mm, 5 µm). Acidified water (0.5% acetic acid, v/v) and acetonitrile were used as mobile phases A and B respectively. Gradient was programmed as follows: 0 min, 0% B; 10 min, 20% B; 15 min, 30% B; 20 min, 50% B; 25 min, 75% B; 30 min, 100% B; 32 min 0% B, and finally, the initial conditions was held for 8 min as a re-equilibration step. The flow rate was set at 0.80 mL/min [31]. A standard solution containing thirteen phenolic compounds (five phenolic acids and eight flavonoids) was injected as a calibration curve and the peak area values were plotted as average values of triplicate injections.

Statistical analysis

Analysis of variance was carried out for all the data sets. One-way ANOVA with Tukey's Honestly Significant Difference test were carried out to analyze the effect of digestates (liquid and solid fraction) on growth parameters (height, collar diameter, leaf, flower and fruit numbers, and leaf area), on DPPH activity and on phenol, ascorbate and flavonoid content. Two way ANOVA was performed to analyze the effects of both digestates, of concentrations and their interaction on plant height, collar diameter, leaf, flower and fruit numbers, and leaf area. All analyses were conducted using SYSTAT 13 for Windows. Significant effects were determined by $\alpha = 0.05$.

Results and discussion

Effects of digestates on growth parameters

Uliva (SU) and Fattoria (SF) solid fractions exerted opposite effects on cucumber growth parameters (Figgs.1-2). Increasing SU concentrations, plant height, leaf number and leaf area were significantly reduced compared to control plants. Collar diameter, fruit and flower numbers were not significantly affected by SU, as well evidenced by statistical analysis (Figgs.1-2). On the contrary, SF at the concentrations of 25 and 50% enhanced collar diameter, plant height, leaf number and especially leaf area, as shown by F-ratio values (Fig. 1).

Liquid fractions of Fattoria (LF) and Uliva (LU) increased leaf number and leaf area (Figgs 1-2) and with a concentration of 10%, the number of flowers in cucumber plants redoubled compared to control (Fig. 2). LF, at the highest concentration, enhanced significantly leaf area with an increase of 78% over the

control (Fig. 2). Plant height and leaf number were positively affected by LF up to a concentration of 20%. F-ratios confirmed that the growth parameters mostly influenced were in the order: plant height> leaf area> leaf number (Figgs. 1- 2). The Uliva digestate induced different and opposite effects on growth, depending on the fraction used (solid or liquid), conversely the effect of Fattoria was mainly due to the concentrations.

In respect to the solid fractions, the results of two-way ANOVA (Fig. 3) suggested that the type of digestate (Uliva or Fattoria) rather than the concentration, induced major changes on plant growth, except for leaf area that was mainly influenced by concentrations, by fractions and by their interaction. Differently, the effects of liquid fractions were mainly related to the concentration rather than to the type of digestate, except for plant height and fruit number that were mostly influenced by the type of digestate (Fig. 3). The combined effect of concentration and digestate was always the least significant, suggesting that there was not a great additive or synergistic effect on growth parameters (Fig. 3). The results on performance of cucumber plants showed that Fattoria induced the best growth responses, confirming previous results of Panuccio et al. (2016), showing that Fattoria digestate with high nutrients, lower phenols and pollutant load, was less phytotoxic on seed germination of cucumber than Uliva.

Effects of digestates on phytochemicals

As demonstrated by many authors, the overall quality characteristics of the plant parts used as food are the result of the interactions among genotype, environmental conditions, cultural practices and postharvest handling and processing techniques (Ceglie et al., 2016; Sekara et al., 2017). Cultural practices and more specifically, organic production systems with an insufficient supply of mineral nitrogen are considered stressful condition, and may induce in plants elevated levels of phenols and ROS as natural defense substances.

Digestate application induced significant changes on antioxidant content in cucumber in comparison with untreated ones. Solid fractions of both digestates, up to the concentration of 50%, increased the amount of total phenols, ascorbate and total antioxidant capacity (DPPH) respect to the controls (Fig. 4). The Flavonoid content was reduced, when both solid fractions were used at concentrations higher than 25%. Despite the similar trend of solid fractions, SF showed a more significant effect (F-ratios) on ascorbate and flavonoid content compared to SU, which conversely caused more changes than SF in phenols and in total antioxidant activity (Fig. 4). The different effects of solid and liquid fractions of both digestates on the antioxidant content strictly depended on the concentrations used. Both liquid fractions significantly increased DPPH activity, ascorbic acid and phenol content, the latter in a concentration dependent manner

(Fig. 4). Flavonoid amount enhanced only in fruits treated with 20% of LF and then progressively decreased. These results are in agreement with findings of Young et al. (2005), showing the positive effects of organic fertilization on plant secondary metabolites of a controlled crop production. Digestate applications significantly affected antioxidant activity of cucumber fruits with an increase in the levels of individual flavonoids and phenols in comparison with untreated ones. This suggested that the addition of digestate, rich in carbon and phenols, stimulated plant-resource reallocation from primary metabolism to secondary metabolite production, driving the synthesis of important health-promoting phytochemicals. Our results are in agreement with previous studies reporting elevated levels of secondary metabolites in organic carrots (Sharma et al., 2012), sweet peppers (Del Amor et al., 2008), and tomatoes (Pieper and Barrett, 2009). Other authors found an increase in phenolic acids in eggplant cultivated with organic techniques (Luthria et al., 2010), higher values of antioxidant activity in organic tomato and bell peppers (Chassy et al., 2006) and high amount of ascorbic acid, α-tocoferol, and β-carotene in organic amended fruits (D' Evoli et al., 2010). Variations of individual phenols and flavonoid evidenced that vanillic acid was present only in treated fruits, chlorogenic acid was the most abundant phenolic acid in control, and its content significantly decreased by increasing the concentrations of both fractions of the two digestates. Ferulic acid was greater in fruits of cucumber treated with the lowest concentration of SU, SF and LF respect to control (Table 1). Among flavonones, nehoesperidine and hesperetin were present only in treated fruits. The rhamnetin concentration in digestate-amended fruits was lower than in control and it decreased when the concentrations of both digestates increased (Table 1). Naringine in treated fruits was always more than in control. In short, treated fruits contained the greatest amount of total phytochemicals and in particular of vanillic acid, naringine, nehoesperidine and hesperetin (Table 1), compounds with considerable antioxidant activity and antihypertensive potential (Kumar and Panday, 2013). Hesperetin, has well-known antioxidant, anti-inflammatory and anti-carcinogenic properties. Naringin and rhamnetin with well-known antioxidant, anti-inflammatory and antiviral properties were the highest in fruits of treated plants. The flavanone glycoside naringin, acting as a free radical scavenger and antioxidant (Naderi et al., 2003; Kumar and Pandey, 2013) is able to reduce the total cholesterol level, the risk of atherosclerosis, the oxidative stress and inflammatory response, enhancing at the same time lipid metabolism. Similar results were recorded by Heimler et al. (2006) in broccoli amended with bio-fertilizer. An increase in kaempferol and quercetin, important flavonols, were also found in cucumber fruits of plants amended with digestates. The increase in flavonoid and phenol contents in cucumber could be related to the organic fertilizers able to induce the acetate shikimate pathway, as already

241 demonstrated by Sousa et al. (2008). Another possible explanation is related to a higher pathogenic
242 content of organic amendment, which in turn can cause biotic stress with a consequent increase in phenols
243 and flavonoids in plant organically grown (Young et al., 2000). These results confirm the anaerobic
244 digestate is suitable for a sustainable and environmental sound agriculture. Moreover, even if the use of
245 digestates in crop management in some case does not foster the productivity, can lead to an advantage of
246 quality by enhancing levels of some health promoting compounds in crop.
247

248 **Conclusion**

249 In short, as the world must now strive to double agricultural productivity, whilst minimizing greenhouse
250 gas emissions, protecting the environment and producing quality food, the use of digestate as organic
251 fertilizer for a sustainable agriculture seems to be a good option for the production of high-quality food,
252 with reduced chemical inputs. The recycle of wastes represents nowadays a key factor for improving soil
253 productivity, plant performance and farm bio-economy, producing high-quality crop with reduced
254 chemical inputs.

255 **Conflict of interest**

256 No conflict of interest occurs

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Figure captions

Figure 1. Effects of both fractions of Uliva and Fattoria digestates on: height, leaf number and collar diameter of cucumber plants. Values are means ± SE (n=3). Lower-case and upper-case letters refer to differences within each digestate treatment. Means followed by different letters are significantly different (Tukey's test at P<0.05). Significance: ***p<0.001; ** p<0.01; *p<0.05.

Figure 2. Effects of both fractions of Uliva and Fattoria digestates on: flower and fruit number and leaf area of cucumber plants. Values are means ± SE (n=3). Lower-case and upper-case letters refer to differences within each digestate treatment. Means followed by different letters are significantly different (Tukey's test at P<0.05). Significance: ***p<0.001; ** p<0.01; *p<0.05

Figure 3. Schematic representation of analysis of variance (Two-way ANOVA) on cucumber growth parameters. Histograms show the effects due to digestates, concentrations (Conc) and their interactions (Conc x Dig) as a percentage of the explained variation. Significance: ***p<0.001; ** p<0.01; *p*p<0.05.

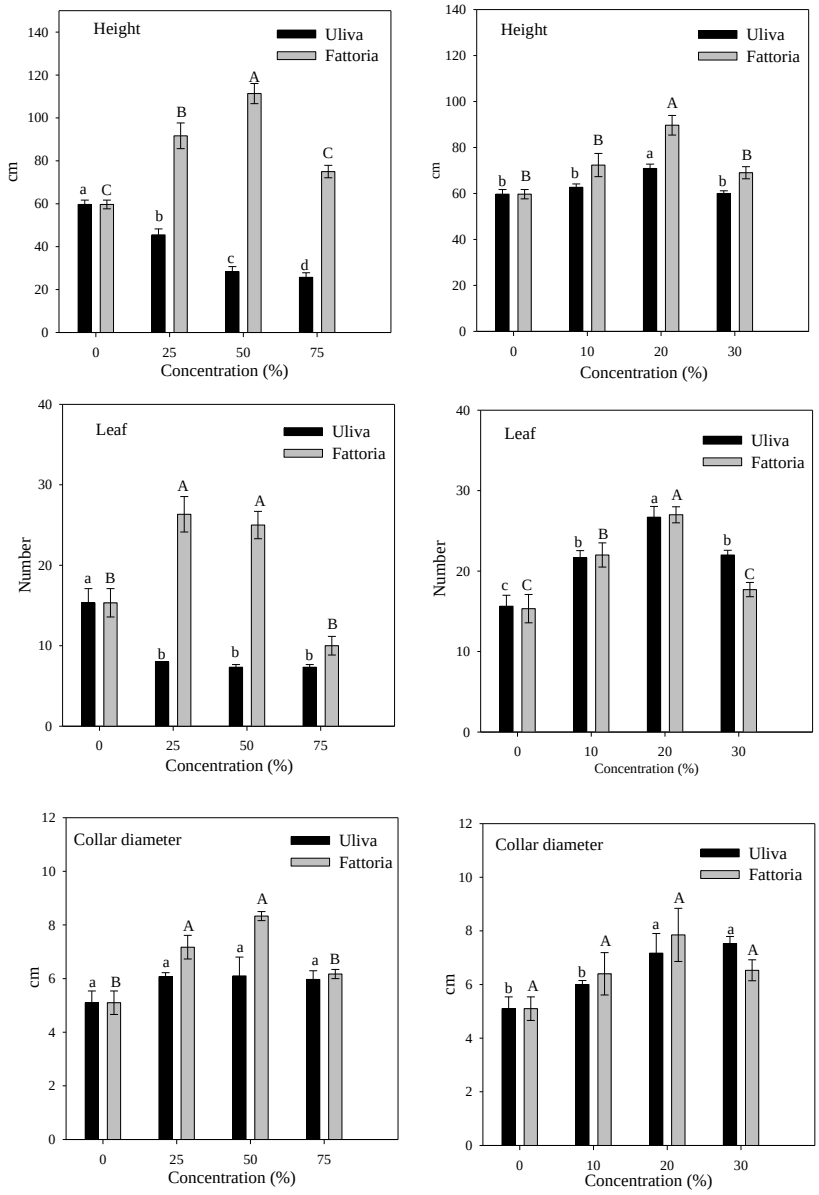
Figure 4. Effects of solid and liquid fractions of Uliva and Fattoria digestates on total phenols, DPPH, ascorbic acid and flavonoid content. Values are means ± SE (n=3). Lower-case and upper-case letters refer to differences within each digestate treatment. One-way analysis of variance (ANOVA) and Tukey multiple comparison tests were performed to compare all pairs of means. Significance: ***p<0.001; ** **p<0.01; *p<0.05

Table 1. Phenolic acids, Flavones and Favonones (mg/L) in cucumber fruits amended with different concentrations of both fractions of Fattoria and Uliva digestates. Control, CTR; Vanillic acid, VAN; Syringic acid, SYR; Ferulic acid, FER; Chlorogenic acid, CHL; Quercetin, QUER; Kaempferol, KAEM; Narirutin, NARR; Naringin, NARG; Esperidin, ESP; Neohesperidin, NEO; Hesperetin, HES; Rhamnetin, RHA; Total content, TOT (mg/100g F.W.) all data were means of triplicate measurements.*nd= below the detection limit. Means with the same letters, in the same column, are not significantly different (Tukey's test. $p \leq 0.05$).

Fattoria													
	Phenolic acids				**Flavones**		**Flavanones**						
	VAN	**SYR**	**FER**	**CHL**	**QUER**	**KAEM**	**NARR**	**NARG**	**ESP**	**NEO**	**HES**	**RHA**	**TOT**
CTR	nd*	0.096^{a}	0.009^{c}	2.042^{a}	0.221^{a}	0.06^{d}	0.203^{a}	0.203^{d}	0.435^{a}	nd	nd	0.543^{a}	260.1^{b}
SF 25%	0.129^{b}	0.032^{b}	0.036^{b}	2.033^{a}	0.218^{a}	0.55^{b}	0.173^{b}	0.289^{b}	0.070^{b}	0.071^{b}	0.367^{a}	0.287^{d}	270.4^{b}
SF 50%	0.074^{c}	nd	nd	1.994^{a}	0.220^{a}	0.78^{a}	0.149^{c}	0.241^{c}	nd	0.045^{c}	0.373^{a}	0.334^{c}	264.9^{b}
SF 75%	0.019^{d}	0.025^{b}	nd	nd	0.218^{a}	0.22^{c}	nd	0.214^{d}	0.031^{c}	nd	0.364^{a}	0.445^{b}	104.9c
LF 10%	0.456^{a}	nd	0.020^{b}	2.052^{a}	0.220^{a}	0.78^{a}	nd	0.388^{a}	nd	0.148^{a}	0.367^{a}	0.264^{d}	312.6^{a}
LF 20%	0.109^{b}	nd	0.069^{a}	nd	0.222^{a}	0.89^{a}	0.142^{c}	0.393^{a}	0.062^{b}	0.065^{b}	0.370^{a}	0.323^{c}	128.3^{c}
LF 30%	0.027^{d}	0.028^{b}	nd	nd	0.221^{a}	0.43^{b}	nd	0.394^{a}	0.087^{b}	nd	0.365^{a}	0.365^{c}	115.4^{c}

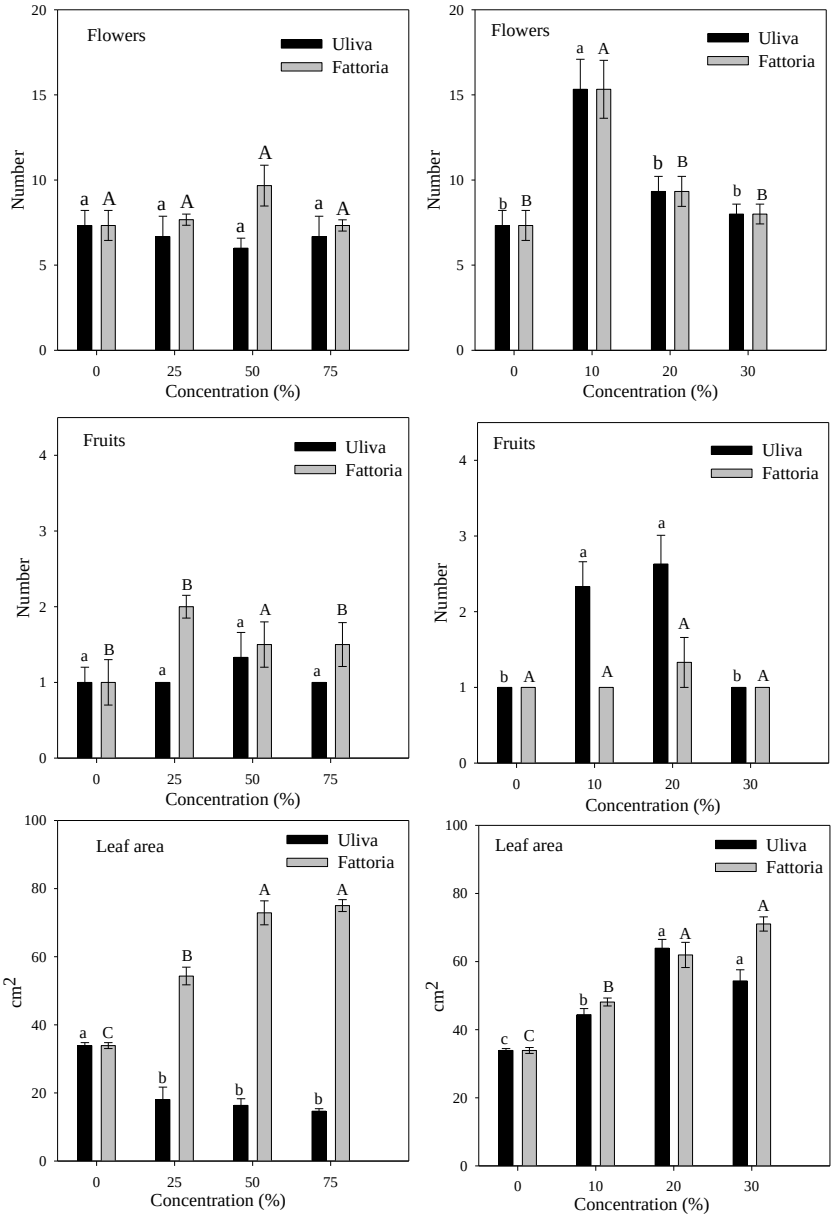
Uliva													
	Phenolic acids				**Flavones**		**Flavanones**						
	VAN	**SYR**	**FER**	**CHL**	**QUER**	**KAEM**	**NARR**	**NARG**	**ESP**	**NEO**	**HES**	**RHA**	**TOT**
CTR	nd	0.096^{a}	0.009^{b}	2.042^{a}	0.221^{a}	0.06^{d}	0.203^{b}	0.203^{e}	0.435^{a}	nd	nd	0.543^{a}	260.1^{b}
SU 25%	0.118^{b}	0.029^{b}	0.034^{a}	2.030^{a}	0.213^{b}	0.48^{b}	0.168^{c}	0.274^{c}	0.078^{b}	0.068^{b}	0.359^{a}	0.281^{d}	266.5^{b}
SU 50%	0.067^{c}	nd	nd	1.987^{a}	0.210^{b}	0.66^{a}	0.169^{c}	0.239^{d}	nd	0.041^{b}	0.365^{a}	0.345^{c}	264.1^{b}
SU 75%	0.016^{d}	0.026^{b}	nd	nd	0.207^{b}	0.09^{d}	nd	0.210^{e}	0.047^{c}	nd	0.361^{a}	0.461^{b}	104.9^{c}
LU 10%	0.370^{a}	nd	nd	2.064^{a}	0.223^{a}	0.53^{b}	0.324^{a}	0.302^{b}	nd	0.134^{a}	0.365^{a}	0.301^{d}	317.6^{a}
LU 20%	0.111^{b}	nd	nd	nd	0.213^{b}	0.33^{c}	0.162^{c}	0.233d	nd	0.050^{b}	0.361^{a}	0.319^{d}	117.7^{c}
LU 30%	0.021^{d}	0.022^{b}	nd	nd	0.216^{b}	0.08^{d}	nd	0.376^{a}	0.082^{b}	nd	0.357^{a}	0.363^{c}	110.3^{c}

SOLID LIQUID

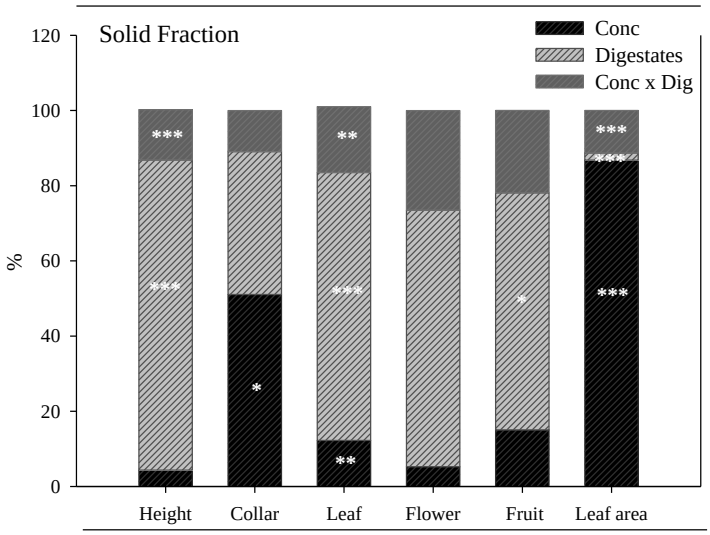


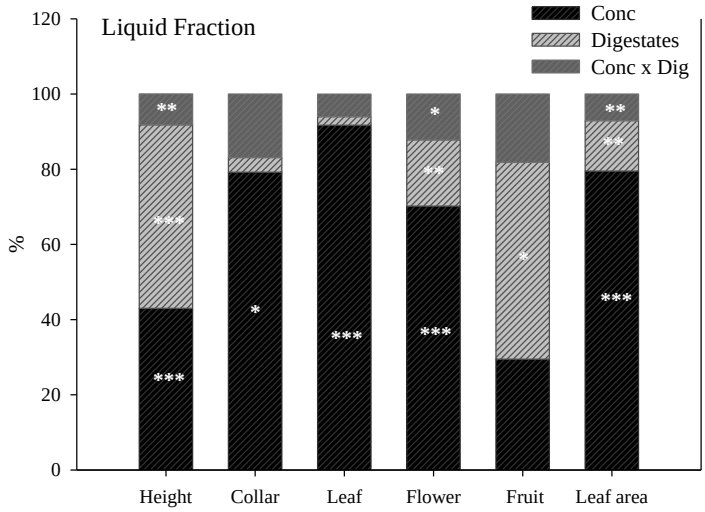
	SU	LU	SF	LF
Height				
R^2	0.945	0.783	0.892	0.812
F-ratio	45.45***	9.63**	22.02***	11.50**
Leaf				
R^2	0.873	0.811	0.871	0.864
F-ratio	18.27**	11.47**	17.99**	16.98**
Collar diameter				
R^2	0.297	0.686	0.94	0.537
F-ratio	n.s.	5.82*	41.51***	n.s.

SOLID LIQUID



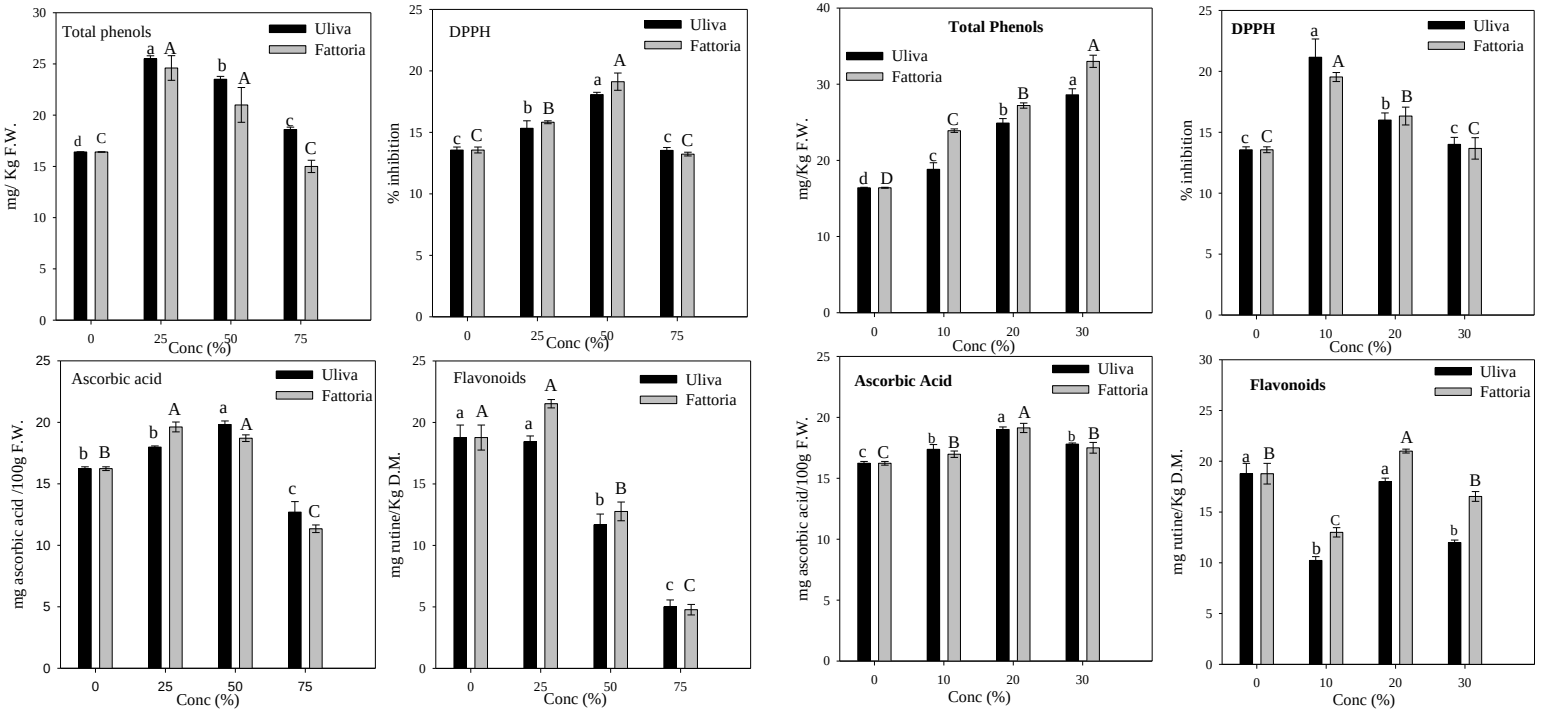
	SU	**LU**	**SF**	LF
Flower				
R^2	0.100	0.874	0.415	0.800
F-ratio	n.s	18.53**	n.s.	10.67**
Fruit				
R^2	0.273	0.500	0.714	0.273
F-ratio	n.s	n.s	6.67*	n.s
Leaf area				
R^2	0.866	0.853	0.987	0.95
F-ratio	17.19**	15.46**	196.07***	51.15***


Solid Fraction
Conc
Digestates
Conc x Dig
%
120
100
80
60
40
20
0
Height
Collar
Leaf
Flower
Fruit
Leaf area


Liquid Fraction
Conc
Digestates
Conc x Dig
%
120
100
80
60
40
20
0
Height
Collar
Leaf
Flower
Fruit
Leaf area

Solid Fraction

Liquid Fraction



SOLID		Phenols	DPPH	Ascorbic Acid	Flavonoids
Fattoria		***	***	***	***
	F-ratio	24.09	44.31	172.05	113.38
	R^2	0.900	0.943	0.985	0.977
Uliva		***	***	***	***
	F-ratio	192.28	104.46	43.86	71.33
	R^2	0.986	0.975	0.943	0.964

LIQUID		Phenols	DPPH	Ascorbic Acid	Flavonoids
Fattoria		***	***	**	***
	F-ratio	417.49	21.14	14.49	56.21
	R^2	0.994	0.888	0. 485	0.955
Uliva		***	**	***	***
	F-ratio	111.94	17.81	27.26	55.49
	R^2	0.977	0.870	0.911	0.954