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Research Article

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Modification of the Phenolic and Fatty Acid Content in *Olea europaea* Olives from Sardinia as a Consequence of *Bactrocera oleae* Infestations

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Abstract

Bactrocera oleae, the main pest of *Olea europaea* fruits, was the focus of our study. We investigated how its infestation influences the biochemical composition of olives from the Nera di Gonnos and Pitz'e Carroga cultivars, emphasizing their potential use in the table olive industry.

Olives were dissected under a microscope to assess the level of infestation and the presence of pupae. The fatty acid composition was studied by GC-FID (gas chromatography, flame ionization detector) and confirmed by GC-MS (gas chromatography-mass spectrometry). The composition of single phenols was determined by HPLC-DAD (liquid chromatography—diode array detector), and total phenols were determined using a UV (ultraviolet) spectrophotometer.

The analysis revealed a relatively low infestation level (below 10%), attributed to the agricultural practice adopted in the orchards. The levels of fatty acid and phenols were almost similar among healthy and infested samples, with less-infested olives (NGD2 and PC) showing higher values of monounsaturated (MUFA), lower saturated (SFA), and polyunsaturated fatty acid (PUFA). Multivariate statistical PLS-DA analysis of fatty acid distinguished healthy and infested fruits. Single phenols had higher amounts of oleuropein and luteolin precursor in healthy than infested olives. Total biophenols expressed as the sum of single phenols showed higher values in healthy vs infested and were positively correlated with the total number of stings, with a higher level of decrease in the samples most infected.

Keywords: Table olive; Phenolic compounds; *Bactrocera oleae*; Fatty acids

Introduction

Olea europaea is an evergreen tree ubiquitous in the Mediterranean area. Olive oil and processed table olives are its main edible products. They are recognized as functional foods thanks to their high level of natural antioxidants and their high-quality composition of fatty acids [1,2]. Epidemiological studies have shown the essential nutritional role of *Olea Europaea* fruits in the Mediterranean diet and the beneficial effect of their components [3]. This property has been attributed mainly to the antioxidant and free radical-scav-

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enging activity of polyphenols contained in olive fruits and olive oil [4-6]. The Mediterranean population enjoys a healthy lifestyle, consuming, on average, 20 times more olive oil than Americans; their cancer risk and cardiovascular disease decrease despite the high animal fat intake [7-9]. The American Heart Association has defined recommendation guidelines reporting an association between the Mediterranean-type dietary models and cardiovascular disease decrease [10].

The phenolic fraction of olives, part of the so-called minor compounds of *Olea europaea* fruits, account for 1-14% of dry pulp and can vary both in quality and quantity depending on several factors, such as genetic, ripening degree of fruits, processing technologies, parasite infestation, and storage [11,12]. The major phenolic compounds identified and quantified in olive and olive oil belong to three different classes: simple phenols (hydroxytyrosol, tyrosol); secoiridoids (oleuropein, the aglycone of ligstroside and their respective decarboxylated dialdehyde derivatives) and the lignans [(+)-1-acetoxy pinoresinol & (+)- pinoresinol] [1,2].

Oleuropein glycoside, the glycoside ester of 2-(3,4-dihydroxyphenyl) ethanol (hydroxytyrosol) with the oleosidic skeleton (exocyclic 8,9-olefinic functionality, a combination of elenolic acid and a glucosidic residue), is the main phenolic compound in olive fruit and is responsible for the bitterness of fresh green olives. Oleuropein glycoside has shown bactericidal and bacteriostatic activity [13] and is not detected in the olive fruit harvested at maturity since it is deglycosylated by glycosidase enzymes, releasing the free secoiridoids [11,12] and is finally hydrolyzed in hydroxytyrosol and elenolic acid [14].

The olive fruit fly (*Bactrocera oleae* Gmelin, 1790) represents the most harmful parasite affecting olive quality. The premature fruit drop represents the primary damage, followed by the deterioration of the visual and texture quality of the olives, making them unusable for processing into table olives. The quality parameters most affected are acidity, peroxide value, ultraviolet (UV) absorbance, and organoleptic quality; negatively alter the chemical composition (sterols, phenols, fatty acid, and volatile fraction) and reduce oil yield [15-18]. The severity of the adverse effects depends on the stage of the development of the olive fly, the intensity of the attack, and the olive variety, leading to a decrease in the phenolic content and the total amount of volatile compound. On the other hand, no significant variation was observed in the fatty acid composition [19,20]. Medjkouh et al. [21] reported a decrease in the phenolic content of two cultivars from Algeria attacked by *Bactrocera oleae*; in contrast, Medjkouh et al. [19] studying eight olive cultivars from Algeria reported an uneven behavior on the composition of the fruits related to the attack. Moreover, no significant variations can be observed in the fatty acid composition [22,23]. Although there are numerous publications about the influence of *Bactrocera oleae* on the qualitative parameters of olive oil, potential variations in the phenolic profile have yet to be considered in depth when considering the preparation of table olives.

This investigation is significant as it aims to investigate the changes in the biochemical composition of olives from the Nera di Gonnos and Pitz'e e Carroga cultivars collected from different regions of Sardinia. We specifically relate the total and single phenolic compounds and fatty acid composition to the percentage of olive fly attacks, highlighting the potential impact of infestation on olive quality.

Materials and Methods

Chemicals and reagents

Tyrosol, 3-hydroxytirosol, luteolin 7 glucoside, luteolin, oloeuropein, verbascoside, p-cumaric acid, vanillic acid, and squalene were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

Stock standard solutions of the analytes were prepared in methanol (1000 µg/mL). Intermediate stock standard solutions were prepared at 100 µg/mL in methanol by dilution of stock standard solutions.

Working standard solutions were prepared in methanol and were used for qualitative and quantitative analysis. The marine oil FAME mix analytical standard was purchased from Restek (Bellefonte, PA).

Sodium hydroxide and phosphoric acid were purchased from Merck (Darmstadt, Germany); sodium carbonate, potassium hydroxide, magnesium sulfate anhydrous, and Folin Ciocalteau reagent were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Double-deionised water with a conductivity of less than 18.2 MΩ was obtained with a Milli-Q system (Millipore, Bedford, MA, USA).

Methanol, ethyl acetate, hexane, and acetonitrile were analytical or HPLC grade (Sigma-Aldrich Inc, St. Louis, MO, USA).

Olive samples

Samples (6 Kg) from the cultivar "Nera di Gonnos" (NG) and Pitz'e e Carroga (PC) were collected within a week in November from four different growing areas of Sardinia: Dolianova 39°21'27.74"N/9°10'50.23"E (NGD1), and 39°23'27.82"N/9°10'49.85"E (NGD2, and PC); Villasor 39°23'21.67"N/8°51'41.51"E (NGV), and Gonnosfanadiga 39°30'4.13"N/8°40'4.81"E (NGG). Samples were collected randomly with three replicates for each area and immediately carried in the laboratory; the olives were separated between non-infested and infested. The degree of olive fly infestation was evaluated by analyzing with a stereomicroscope (Zeiss, Italy) dissection sample from 100 olives randomly selected from the main bulk. The number of oviposition scars (stings), live larvae, and pupae or larval/adult exit holes was determined.

Fatty Acid Composition: Three g of homogenized olives were extracted with 10 mL of hexane. Methyl esters were prepared by alkaline treatment, which was carried out by mixing 0.5 mL of the hexane extract with 0.1 mL of 2 N potassium hydroxide in methanol and mixing for 1 min with a vortex alternating heating with stirring, according to Christie et al. [24]. A GC Thermo 8000 TOP, coupled with a flame ionization detection (FID), a capillary column Supelco 24111 – SP™ (60 m x 0.25 µm, film 0.2 µm), was used. Nitrogen was the carrier and make-up gas at 200 KPa and 100 KPa, respectively.

Oxygen and hydrogen were at 95 and 70 KPa, respectively. The injector and detector temperatures were 220 and 250 °C, respectively. The oven program was as follows: T=0 90 °C (1 min.), till 100°C (2 °C/min) held 3 min, till 245 °C (3 °C/min) held 20 min. GC-MS confirmed the methyl esters. A gas chromatography-mass spectrometry Thermo DSQ (GC-MS DSQ, Thermo Finnigan, Milan, Italy) with a Select Fame column (Agilent Technologies, 100 m, 0.25mm, 0.2 μm) was used. GC conditions were T=0 100 °C (1 min.), till 160 °C (3 °C/min) held 3 min, till 198 °C (1 °C/min), till 250°C (5 °C/min) held 15 min. The injector and transfer line were at 250 °C.

MS analysis was carried out in EI+ with an m/z scan rate from 50-500 amu. Computer matching against a commercial library [25] and homemade library mass spectra made from pure substances and MS literature data were used to identify.

Phenolic Fraction: Phenolic compounds were extracted from olives according to the method for olive oil of COI [26] with some minor changes. Three grams of homogenized olives were extracted twice with 15 mL of methanol/water (80/20, v/v) solution and 10 mL of hexane. The tubes were agitated for 20 min in a rotatory shaker, and the organic layer was separated. The two extracts were combined, filtered through a 0.45 μm PTFE syringe filter (Whatman Inc., Clinton, NJ, USA), and dried in rotavapor (t= 30 °C). The residue was dissolved in 15 mL of ethyl acetate plus 2 g of anhydrous $MgSO_4$ to remove the remaining water fraction. One mL of the ethyl acetate solution was dried under a gentle nitrogen stream, recollected with 1 mL of methanol, and injected in HPLC for the analysis. An HPLC 1100 (Agilent Technologies, Milan, Italy) coupled with a DAD detector UV 6000 (Thermo Finnigan, Milan, Italy). The column was a Varian Polaris C18 (5 um, 300 A, 250 mm x 4.6 mm). The analysis was carried out at λ 280 and 360 nm in gradient elution. Solvents were phosphoric acid 0.22 M (A), acetonitrile (B), and methanol (C). The gradient used for the separation and analysis was: T=0 A 96%, B 2%, C 2%; T=40 A 50%, B 25%, C 25%; T=45 A 40%, B 30%, C 30%; T=60 A 0%, B 50%, C 50%, hold 10 min; post time 15 min. Flow 1 mL/min. Calibration graphs were prepared with five points from 5 to 50 μg/mL.

Total phenol: 10 g of olives were extracted with 20 mL of methanol/water (80/20, v/v) solution for 30 min in a rotatory shaker. The extracting solution was centrifuged for 10 min at 4000 rpm and diluted ½ with methanol before the analysis. Total phenols were determined using the Folin-Ciocalteu method. 100 μL of the diluted extract solution were put in a 10 mL calibrated flask with 500 μL of Folin-Ciocalteu reagent, 3 mL of a sodium carbonate solution 20% (p/v), and MilliQ water till 10 mL. The mixture was agitated for 1 minute in a vortex and incubated for 80 min at room temperature (18 °C) in the dark. Before UV analyses, samples were centrifuged at 4000 rpm for 10 min. A Varian Cary50 spectrophotometer with a 1 cm quartz cuvette, set at λ 725 nm, was used. Quantitative analyses were made using the external standard method, using gallic acid as a standard reference and correlating absorbance to concentration. Calibration graphs were made between 200 and 2000 μg/g; the results were expressed as μg/g of gallic acid.

Statistical Analysis: Data were analyzed using Statistica 6.0 (Statsoft, Tulsa, OK, USA) statistical software. Unless otherwise stated, the reported values are averages of at least three repetitions (n= 3). Tukey's honest significant difference (HSD) multiple comparisons (one-way ANOVA) and Pearson's linear correlations are both at $p < 0.05$.

GC-MS data sets were imported into SIMCA 13 (Umetrics AB, Umea, Sweden) for processing using principal component analysis (PCA) and partial least squares regression (PLS-DA). Additionally, R2 (the multiple correlation coefficient) and Q2 (the cross-validated correlation coefficient) were used as touchstones for the robustness of a pattern recognition model [27].

Results and Discussion

Olive's infestation

Olives were classified as infested when bearing stings and healthy olives. The data showed a generally low infestation of total olives harvested, with higher values for NGV and NGG (8%) and lower for PC and NGD2 (2%). These data are compatible with olive oil production, which tolerates values of infestation ≤ 10%; however, a degree of infestation greater than 1% prevents the use of the fruits as table olives [28]. Among the samples tested, NGV>NGG>NGD1 showed the higher infestation, while PC and NGD2 showed the lowest (Table 1). NGV samples were the most affected, with 597 total stings, a maximum number of stings for olives of 47, and 30% with stings ≥ 7. NGV was the only sample showing larvae L2 and L3, accounting for 19 total exit holes. The samples NGG and NGD1 had 572 and 470 total stings, maximum stings, and exit holes of 18 and 10 and 21 and 10, respectively (Table 1).

The percentage of olives with stings ≥ 7 was 32, and 25% for NGG and NGD1, respectively. The samples of NGG showed an old infestation with the absence of larvae or pupae in the pulp but 21 exit holes, while two pupae were detected in the samples NGD1 and NGV. The samples of PC and NGD2 showed the lowest level of olive fly attack with only 297 and 243 total punctures and 6 and 5 exit holes, respectively. The maximum number of stings per olive was 7 and 8, with 2% of olives with stings ≥ 7. No larvae or pupae were detected in PC and NGD2 samples (Table 1).

Table 1: Infestation parameters of the olives collected during the experiment.

	NGD1*	NGD2	NGG	NGV	PC¥
% infestation	6	2	8	8	2
total stings	470	243	572	597	297
% stings/olive b ≥7	25	2	32	30	1
average stings ($\bar{x}$) ± RSD%	5.1 ± 2.4	2.6 ± 1.6	6.2 ± 3.4	6.6 ± 5.4	3.2 ± 1.3

stings max-min	10 - 1	8 - 1	18 - 1	47 - 1	7-1
L2	0	0	0	1	0
L3	0	0	0	1	0
pupae	2	0	0	2	0
exit holes	10	5	21	19	6

* NG – Nera di Gonnos

¥ PC – Pitz'e e Carroga

Olive fruit fly adults exhibit, under field conditions, a preference for large fruit [20,29]. Olives of the cv Nera di Gonnos (NG) and Pitz'e Carroga (PC) have similar weights (around 5 g) but different shapes; NG is elliptic, while PC is asymmetric with a sharp tip (Figure 1). PC is susceptible to *Bactrocera oleae* because of its size and earliness.

Figure 1: Differences in the shape of olives from the cultivars Nera di Gonnos and Pitz'e Carroga.

The samples of NGD2 and PC belonged to the same orchard and were subjected to the same field management. The plants followed a meticulous protocol of treatments with plant protection products, which decreased the population of *Bactrocera oleae* and, consequently, fly attack in these samples. The resulting low level of damage on the olives confirms this behavior.

Fatty acid composition

The chromatographic conditions allowed an excellent separation of the fatty acids methyl esters in the analyzed samples (Figure 2).

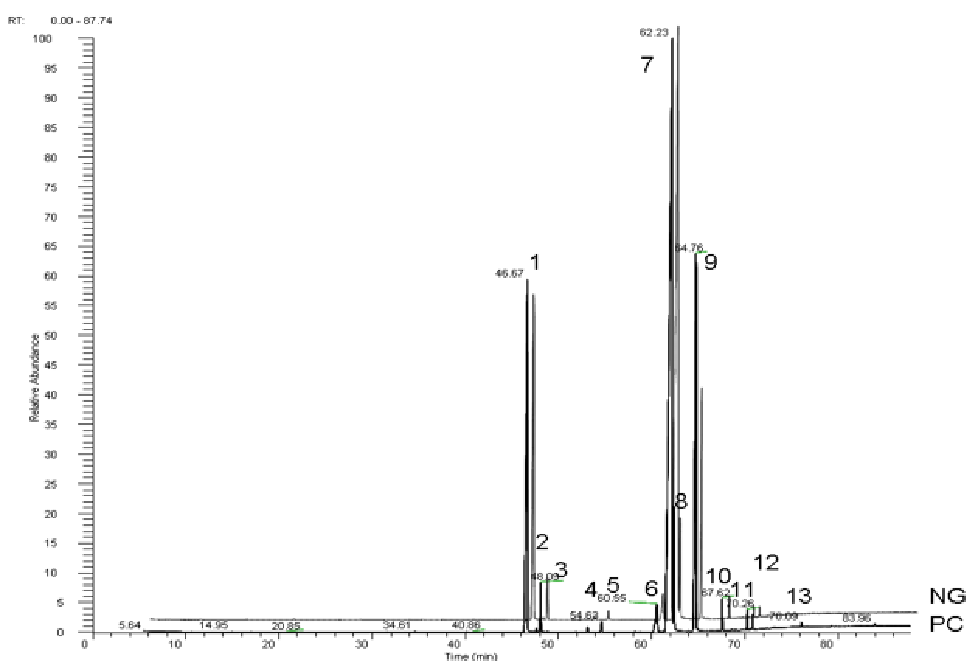


Figure 2: Gas-chromatography MS chromatograms of fatty acid methyl esters from olives of the cv Nera di Gonnos (NG) and Pitz'e Carroga (PC).
1) palmitic acid, 2) cis-7 hexadecenoic acid, 3) palmitoleic acid, 4) eptadecanoic acid, 5) cis-10 eptadecenoic acid, 6) stearic acid, 7) oleic acid, 8) vaccenic acid, 9) linoleic acid, 10) arachic acid, 11) γ -linolenic acid, 12) α -linolenic acid, 13) cis 13.16 docosadienoic acid.

Thirteen fatty acids were detected and subsequently identified by GC-MS. No qualitative differences were observed among healthy and infested olives, neither among different samples of the same cultivar nor from cultivars.

Concerning data, little differences were observed in the quantitative composition of fatty acid among olive samples from healthy or infested cultivars. Oleic acid was the most concentrated methyl ester in all samples, with average values of 56.84 ± 3.33% and 56.38 ± 4.99 in healthy and infested, respectively, followed by linoleic acid (15.14 ± 11.27% healthy and 14.52 ± 27.06% infested), palmitic acid (18.30 ± 4.49% healthy, and 18.58 ± 3.64 infested), all other fatty acids accounted for less than 4% (Table 2).

However, comparing healthy and infested samples from each orchard, we identified two patterns: NGD1, NGG, NGV, and PC and NGD2 (Table 2).

Table 2: Fatty acid composition (%±RSD%) in the samples of healthy and infested olives collected in Sardinia during the experiment.

Acid Methylester	NGD1		NGD2		NGG		NGV		PC	
	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
Palmitic	17.60±0.48	18.30±1.15	17.33±1.70	17.67±3.09	18.87±2.21	18.48±2.86	18.47±4.16	19.14±9.27	19.18±1.14	18.36±2.91
cis-7 hexadecenoic	0.07±1.57	0.08±1.44	0.10±3.56	0.08±5.63	0.10±4.49	0.11±2.82	0.08±29.95	0.08±5.43	0.08±3.67	0.07±17.68
Ac. Palmitoleic	1.46±0.96	1.64±1.05	1.51±2.30	1.39±4.09	1.36±3.59	1.52±3.65	1.59±7.98	1.77±4.29	1.79±1.89	1.57±2.39
Ac. Eptadecanoic	0.11±0.30	0.11±0.58	0.14±2.91	0.14±2.02	0.12±1.79	0.12±5.89	0.14±5.59	0.15±4.99	0.09±3.06	0.10±6.85
Ac. cis-10 eptadecenoic	0.26±1.24	0.26±0.76	0.33±3.00	0.38±2.15	0.29±1.62	0.28±1.89	0.37±7.33	0.36±0.48	0.23±2.25	0.27±3.09
Stearic	1.50±0.24	1.50±2.49	1.60±0.59	1.54±0.29	1.52±0.83	1.53±3.22	1.34±10.17	1.40±0.58	1.51±1.34	1.45±5.57
Oleic	58.30±0.37	54.70±0.61	58.48±0.46	61.79±0.83	53.83±0.56	51.00±1.48	57.10±5.35	52.90±1.82	56.51±0.98	61.41±3.06
Vaccenic	3.38±8.22	3.84±4.21	3.44±14.91	3.20±16.51	4.53±8.36	5.40±6.34	4.79±40.71	5.48±11.57	4.35±6.69	4.31±24.20
Linoleic	15.24±0.85	16.61±0.25	15.20±4.03	11.11±1.67	17.82±1.62	19.15±1.64	14.48±3.08	16.20±4.19	13.12±0.09	9.69±5.38
Arachic	0.24±1.75	0.24±1.56	0.26±5.29	0.17±86.87	0.26±1.03	0.25±3.88	0.21±2.52	0.19±9.16	0.26±11.04	0.17±3.24
γ-Linolenic	0.52±1.11	0.65±0.49	0.59±0.52	0.49±0.42	0.71±9.50	0.83±6.79	0.56±8.31	0.65±11.96	0.76±1.24	0.62±6.91
α-Linolenic	0.23±0.64	0.26±3.13	0.28±20.82	0.24±0.25	0.93±4.17	1.05±2.46	0.19±3.84	0.22±2.61	0.29±3.62	0.28±2.29
cis 13.16 docosadienoic	1.14±4.67	0.78±2.40	0.85 ±4.45	0.80±0.01	0.88±3.25	0.66±5.61	2.51±8.96	1.61±21.27	1.83±18.46	1.65±19.46
SFA	19.45± 2.27	20.15±5.26	19.30±2.73	19.55±0.85	20.08±1.04	21.31±1.01	20.19±2.24	20.84±0.81	21.06±2.89	20.11±9.65
MUFA	63.50±0.64	60.48±0.53	62.84±1.03	66.85±0.80	59.98±0.25	57.90±0.54	63.94±0.82	60.61±0.60	62.96±0.65	67.71±1.21
PUFA	17.09±1.89	18.29±3.61	16.82±0.67	12.73±3.06	20.32±1.99	21.64±3.48	17.76±1.86	18.47±1.61	15.95±0.05	12.25±1.66
Oleic/linoleic	3.84±2.22	3.30±3.45	3.87±1.71	5.52±4.10	3.02±2.19	2.67±4.10	3.94±2.66	3.31±2.40	4.31±0.83	6.34±2.86
Squalene	7.14±15.70	8.35±7.32	7.97±9.13	1.04±8.78	2.10±7.98	1.80±7.27	5.47±14.45	6.42±15.88	32.07±9.85	20.71±8.32

The former had higher levels of oleic acid and lower palmitic and linoleic acid in healthy olives, whereas the second had higher palmitoleic and linoleic and lower oleic acid in healthy olives related to infested. All samples showed higher levels of cis 13, 16 docosadienoic acid in healthy vs infested. Montedoro et al. [30] reported that fatty acid composition could be influenced by various factors such as environmental factors, infestation, and agronomic techniques; on the contrary, Mraicha et al. [31] observed that also very severe infestation did not cause essential changes in the fatty acid composition. More recently, Valencic et al. [32] reported that olive oils affected by damaging infestation had lower amounts of oleic acid and higher amounts of myristic, linoleic, and linolenic acids. Our data confirmed the slight differences in the fatty acid composition among healthy and infested olives; however, the results showed a reversed behavior compared to the literature data.

The rate of oleic/linoleic acid showed higher values in the most damaged olives (NGV, NGG, and NGD1) than in the less infested (NGD2 and PC) (Table 2).

Grouping fatty acid for the level of unsaturation evidenced MUFA as the main fraction in all samples, followed by SFA and PUFA. NGD1, NGG, and NGV had average values in infested olives, slightly higher for saturated (SFA) and polyunsaturated (PUFA) and lower values for monounsaturated (MUFA) compared to healthy.

On the contrary, in healthy olives, NGD2 and PC showed lower SFA and PUFA values and higher MUFA values (Table 2) than infested ones (Figure 3). Moreover, the oleic/linoleic acid ratio was almost even in all samples of NG, while it was higher in the infested samples from PC and NGD2.

Fatty acid data were analyzed using multivariate statistical analysis (Figure 4). A PCA model was created using all samples. Healthy samples were identified by the letter "a" while infested by the "b". The scores plot of the principal component 1 versus the principal component 2 confirmed the similarity among the samples (Figure 4A). However, PC samples were well separated from NG samples. NGVb samples were probably distinguished from the others for a higher infestation level.

The PLS-DA model, created to evaluate the significance of the difference between healthy and infested olives, showed poorly differentiated classes. Only NGVb, the most affected by olive fly, was separated from the other samples (Figure 4B).

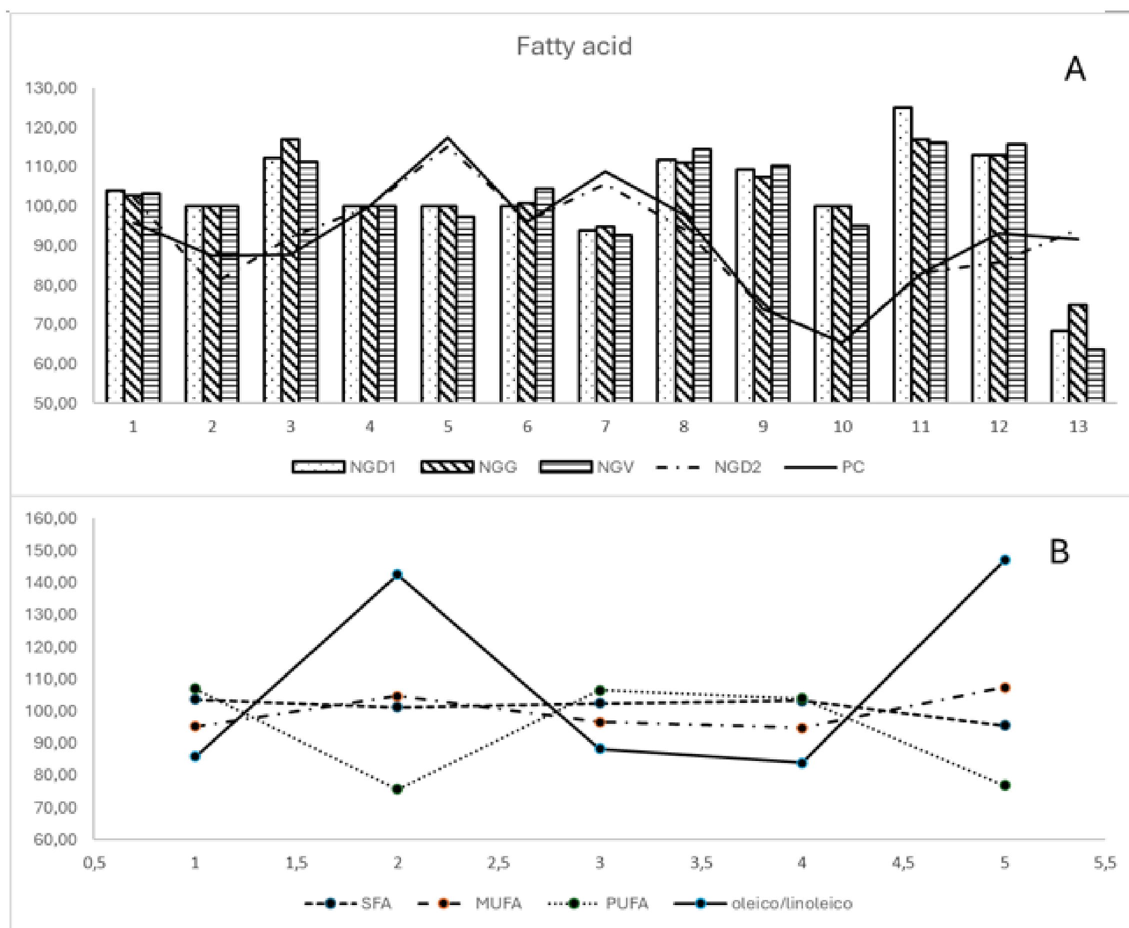
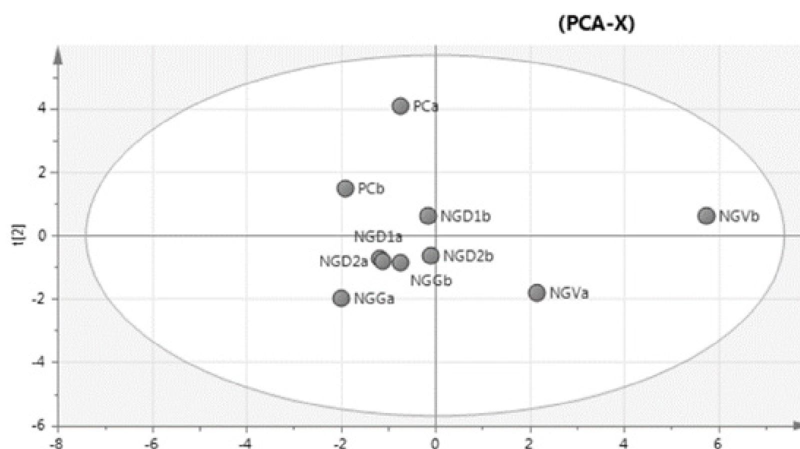


Figure 3: A) Single fatty acid rate % healthy/infested olives, B) Rate % healthy/infested olives grouped for the level of unsaturation.

The importance of the variables in distinguishing the two groups was ranked according to their VIP scores in the PLS-DA model. The results indicated that oleic acid (variable 8) had the highest correlation with the grouping of the infested olives, followed by palmitoleic (variable 3) and linolenic acid (variable 11).



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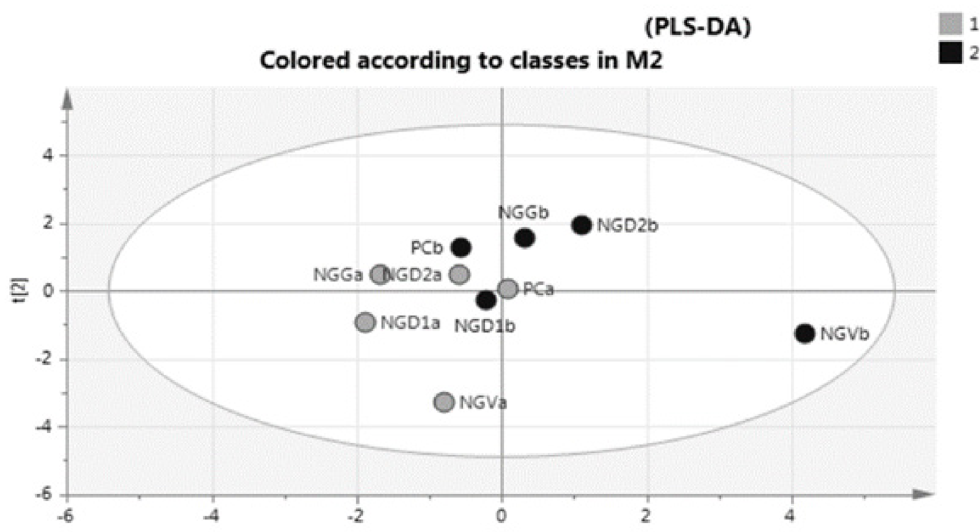

Figure 4: Principal component analysis (PCA) (A) and PLS-DA (B) of fatty acid methyl esters of healthy (a) and infested (b) samples of olives collected during the experiment.

Squalene showed uneven amounts among the different samples. The trend was similar, with lower amounts in infested vs. healthy in NGD2 and PC, while NGD1, NGG, and NGV samples showed an even amount between healthy and infested. PC showed values notably higher, accounting for 32.07 ± 9.85 and 20.71 ± 8.32 in healthy and infested, respectively (Table 2).

Phenolic fraction composition

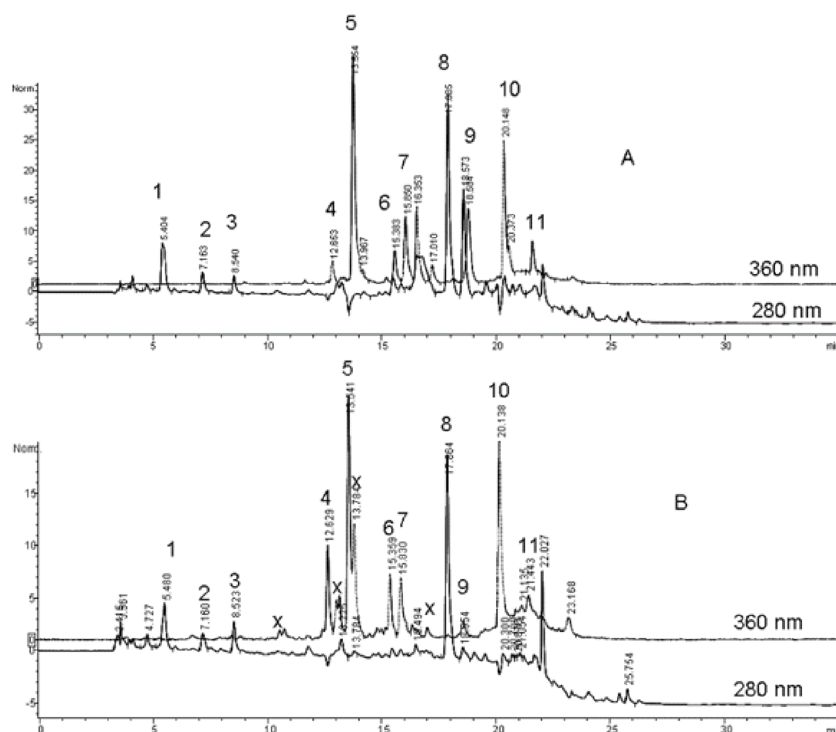

Figure 5: HPLC-DAD chromatograms of the phenol fraction from olive extracts analysed at 280 and 360 nm. A) NG samples, B) PC samples. 1) hydroxytyrosol, 2) tirosol, 3) vanillic acid, 4) p-cumaric acid, 5) luteolin 7-glucoside, 6) verbascoside, 7) 3-OH-cinnamic acid, 8) oleuropein, 9) oleuropein glucoside, 10) CH3-luteolin, 11) luteolin.
X: indicate compounds found in infested samples in both cultivars.

The analysis of HPLC-DAD chromatograms at 280 and 360 nm allowed us to identify 11 main phenols: hydroxytyrosol, tyrosol, vanillic acid, vanillin, p-cumaric acid, luteolin 7-glucoside, verbascoside, 3-OH-cinnamic acid, oleuropein, luteolin, methyl-luteolin (Figure 5). All cultivars belonging to Nera di Gonnos (NG) had overlapped chromatographic profiles (Figure 5A), while PC showed a specific profile at 360 nm with the presence of three undefined peaks and the absence of the peak at 16.35 (Figure 5B).

PC samples showed notably less amounts of Oleuropina glycoside. The 280 nm and 360 nm analyses showed minor differences between healthy and infested samples.

Hydroxytyrosol, tyrosol, luteolin, and CH3-luteolin had lower values in healthy olives samples, while oleuropein, oleuropein glucoside, and luteolin-7-glucoside had higher values in healthy samples (Table 3).

NGV, NGD1, and NGG showed the most remarkable differences between healthy and infested samples (Table 3). At the same time, NGD2 and PC had almost overlapping values except for oleuropein glucoside, luteolin, and methyl-luteolin. The data of total phenols corresponded to the number of stings, with NGD2 and PC almost overlapping (Figure 6). Spectrophotometric analysis of total phenols showed a reversed amount in NGD2 and PC, with less significant differences compared to HPLC DAD analysis.

Table 3: Phenolic composition (μg/g ± RSD%), and total phenols (μg/g ± RSD) in the samples of healthy and infested olives collected in Sardinia during the experiment.

Compounds	NGD1		NGD2		NGG		NGV		PC	
	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
OHTirosol	289.5±5.7	869.7±13.5	402.2±10.8	558.1±11.1	296.6±8.9	788.2±7.8	361.9±5.7	468.3±16.9	345.7±6.1	438.9±14.7
Tirosol	150.7±7.1	347.5±16.8	173.8±13.2	190.7±13.7	125.6±2.2	306.4±12.8	150.8±1.1	230.0±13.6	158.2±12.2	161.7±3.7
Vanillic Acid	122.6±15.1	154.6±4.9	129.4±10.3	149.6±5.7	116.5±0.0	183.6±11.2	171.5±18.5	178.1±11.3	175.0±15.2	189.2±11.7
p- cumaric Acid	24.0±14.4	14.2±9.1	161.0±5.2	211.6±0.5	23.7±4.1	19.5±11.6	25.6±19.4	19.9±10.0	134.0±15.1	92.0±7.3
Verbascoside	251.0±15.7	305.1±7.7	255.3±2.3	239.1±3.7	220.2±14.6	305.6±2.1	189.3±7.8	309.0±11.4	231.7±16.5	353.3±3.1
3-OH idrossicinnamic Acid	56.2±15.6	41.9±7.9	46.6±5.3	45.0±15.8	59.3±0.0	43.3±0.0	55.2±16.6	37.5±10.6	42.6±11.4	46.5±15.6
Oleuropein	817.6±2.4	161.3±2.5	781.6±10.3	534.7±5.3	957.4±1.1	182.3±11.0	1175.3±8.9	130.7±2.6	819.2±2.7	667.0±12.7
Oleuropein glucoside	12324.6±4.8	8589.6±19.0	15490.9±1.7	13332.6±5.6	11504.3±2.2	8479.9±8.5	16250.0±10.6	10717.9±12.4	7803.1±15.4	6682.8±12.6
Luteolin	173.3±7.4	422.6±4.4	154.8±2.5	284.5±10.8	205.9±3.6	304.5±9.8	204.0±8.0	365.2±1.2	430.9±8.9	459.7±10.2
Methyl-luteolin	276.2±10.5	323.8±24.5	269.3±6.2	275.3±8.4	260.5±5.3	391.8±27.7	261.7±12.1	390.9±18.5	70.7±4.8	85.6±12.8
Luteolin 7glu	51.2±9.6	12.7±6.9	57.3±11.7	50.0±3.5	46.1±5.1	12.5±12.1	70.0±8.4	16.2±3.4	54.4±14.7	55.5±2.7
Total phenol DAD	14537.0±13.1	11242.9±8.1	17652.9±8.4	15871.3±10.1	13816.1±7.1	10625.8±7.2	18915.3±13.8	12863.7±12.3	10195.0±14.7	9146.5±9.0
Total phenol as Gallic acid	2774.2±10.1	2857.3±14.2	2771.4±3.7	2588.9±12.1	3013.1±5.6	3178.4±8.4	3428.9±2.9	3868.3±7.8	2427.4±2.6	2327.1±3.4

The two analyses are performed at different wavelengths, and the analytical responses cannot be compared.

Hydroxytyrosol and tyrosol derive from oleuropein and ligstroside hydrolysis, and each process that may cause hydrolysis of oleuropein and ligstroside can increase these two metabolites.

Literature data reported that the infestation of olives by *Bactrocera oleae* can cause extensive damage to the tissue and the cells of the olive pulp, affecting the phenol fraction and the aromatic profile [17,19,32-34].

The data reported in this paper confirmed the general decrease of phenol fraction; a significant decrease of oleuropein and oleuropein glucoside can be noted, with a resulting increase of OHtyrosol and tyrosol.

The damage related to the infestation against the cells increases the release of enzymes, which leads to the degradation of the secoiridoids compounds, growing the amount of the smaller phenols. This condition can be characterized in the samples NGV, NGG, and NGD1, most affected by the parasite (Table 3). A decrease in the total content represents the trend of the bisphenols; however, each single phenol follows a proper dissipation rate.

The rates were well related to the level of infestation; NGD2 and PC showed the lowest differences among the reported phenols.

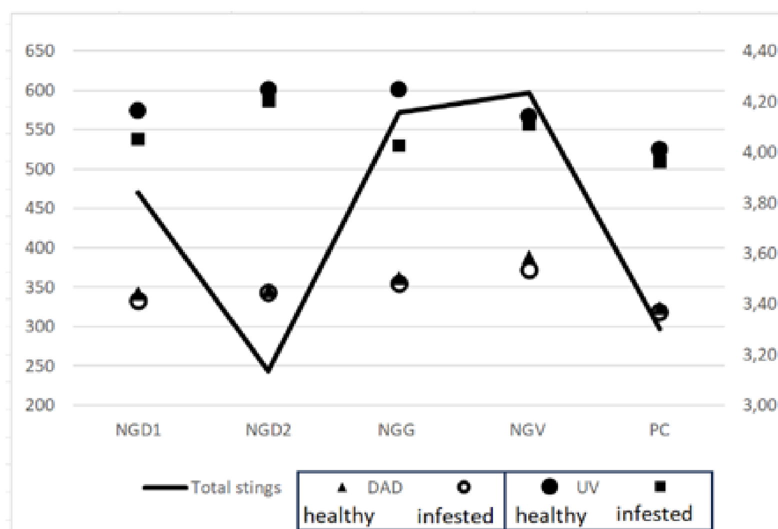

Figure 6: Graphical report of total phenols in healthy and infested olives, calculated by HPLC-DAD as the sum of single phenols identified and total phenol by UV spectrophotometric analysis expressed as gallic acid, compared with the total number of stings.

Conclusion

It has been reported that olive infestation could affect olives and olive oil quality. In this paper, we examined the changes in some olive quality parameters concerning *Bactrocera oleae* infestation. The samples analyzed showed a level of infestation ranging from 10 to 2% and were above the level accepted (1%) for the technological processing of table olives. However, the biochemical analysis showed beneficial characteristics, with only minor differences from healthy olives. The olives most affected by infestation, both for the number of stings and the total level of infestation, were represented by the samples of NGV, NGG, and NGD1. Infestation influenced only slightly the fatty acids fraction; however, it was possible to distinguish two sets of data, constituted by NGG, NGD1, and NGV, which showed higher levels of SFA and PUFA and lower MUFA in healthy vs infested olives. In comparison, NGD2 and PC showed higher MUFA levels and lower SFA and PUFA. The oleic/linoleic acid rate confirmed the presence of two distinguished patterns, which were well separated by multivariate analysis. This fact may indicate a degradative process of MUFA to load in two opposite directions, the increase of unsaturation and the saturation of individuals' double bonds. Single phenols showed higher amounts of oleuropein and luteolin precursors in healthy than infested olives, data confirmed by the analysis of total phenols, which had higher values in healthy vs infested. The differences among single and total phenols (as the sum of single phenols) were positively correlated with the total number of stings, decreasing in the samples most infected (Figure 6). The data demonstrates that new studies are needed to understand better the biochemical reaction at the base of phenols and fatty acid modifications in response to pest attacks.

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None.

Conflict of Interest

No conflict of interest.

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