Influence of olive fly (*Bactrocera oleae*) on the phenolic composition and antioxidant activity of four Algerian olive cultivars

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The olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is one of the main pests of the olive tree which can affect the production and quality of the products. The phenolic compounds are important biological constituents and play a significant role in the susceptibility or tolerance of a cultivar to fly attack. This work aimed to study the influence of the attack of this pest on the phenolic composition and the antioxidant activity of four Algerian olive cultivars. The weight, maturity index, attack rate, phenolic profile, and antioxidant activities (Reducing power, ABTS assay and Chelating capacity) of the olives of the cultivars were determined. Phenolic compounds were determined by HPLC.
The results showed that the size of the fruit (weight) was significantly correlated with the attack (r = 0.91). The phenolic composition was significantly affected; the total losses of polyphenols were maximal in infested olive samples of Ferkani (52.64%) and Souidi (42.71%). Consequently, the antioxidant activities evaluated by different methods decreased significantly, the losses reached 86%. The values of the Relative Antioxidant Capacity Index (RACI), which represent the average scores of the antioxidant activities of each sample, showed that the varieties have different sensitivities. The lowest scores were recorded by attacked olives. The results confirmed the importance of healthy fruit in obtaining products with a high level of phenolic compounds.
Keywords: *Bactrocera oleae,* olives, phenolic compounds, HPLC, antioxidant activity.

1. INTRODUCTION

Olive trees have been growing throughout the Mediterranean basin for between six and seven millennia. During the colonisation period (16-18th centuries) all the regions of the world with a similar Mediterranean-type climate experienced planting by Spanish, Italian or French settlers. It was domesticated as the Oleaster [1, 2] and its cultivation spread to regions where the wild olive tree (oleaster) cannot thrive. They are grown for oil and canned fruit production; very little cultivation has a decorative purpose [3].
Bactrocera oleae (Rossi) (Diptera: Tephritidae), the olive fruit fly, is a key pest of Olea europea particularly in the Mediterranean area where more of the 90% worldwide olive cultivation takes place. This pest can develop 2-5 generations/year, and due to the feeding activity of larval instars on fruits, it is capable of strongly affecting quality and quantity of the olive production [4]. Damages appear during fruiting, when the insect females lay their eggs in the olive fruit pulp and, subsequently, larvae feed and grow in the fruit issues inducing serious losses, both qualitative and quantitative, to the fruit and oil production [5]. During larval development, pulp consumption destroys several tissues in the olive fruit, which leads to a lipolytic reaction between lipases and triacylglycerols, therefore arising the amount of free fatty acids in the olive oil [6].
Moreover, fly infestation increases olive oil acidity and peroxide value, as well as musty and earthy off-flavours, extensively reducing oil quality (e.g., down-

grading extra virgin olive oil to less valuable categories). Indirect effects are mainly due the presence of necrotic areas and microorganisms in feeding tunnels [7].
Tolerance to the olive fly was complex [8]. Many factors are involved: mechanical barriers (e.g., aliphatic waxes), chemical factors (e.g., oleuropein, cyanidin), morphological characteristics (e.g., fruit size) and their combination. Also, the relative importance and contribution of these factors is not yet fully clarified [9 - 11].
Olive (*Olea europaea* L.) fruits contain numerous secondary metabolites, primarily appreciable amounts of phenolic compounds which are particularly interesting for their nutraceutical properties [5]. These antioxidant compounds have numerous human health benefits and are important in the plant defence against pathogens and insects. The objective of this work is to study the relationship between fruit weight and its attack rate by the pest, and to assess the influence of the fruit fly *Bactrocera oleae* on phenolic compounds and antioxidant activity.

2. MATERIAL AND METHODS

2.1. SAMPLING

Olive fruits of *Abani* (A), *Ferkani* (F), *Rougette de Mitidja* (R) and *Souidi* (S) cultivars were collected manually from the trees in the Olive production station in Takarietz (Sidi-Aich, southern Béjaia) in Algeria in 2014 (located at 36°, 36', 47" north and 4°, 41', 18" east, at the altitude of 111m).

2.2. FRUIT WEIGHT

The fruit weight of the studied cultivars was determined as the weight of 100 drupes randomly picked from aliquots of samples previously homogenised [12].

2.3. DETERMINATION OF MATURITY INDEX (MI)

The maturity index is determined according to the formula established [13]. This formula is based on a punctuation system corresponding to each stage of coloration of the pericarp and the mesocarp.

2.4. DETERMINATION OF ATTACK RATE (AR)

The attack rate of samples was determined by calculating the number of olives attacked in a batch of 100 olives taken randomly after harvesting. It is calculated using the formula described [14].

2.5. SORTING AND PREPARATION OF OLIVE SAMPLES FOR THE DIFFERENT ANALYZES

After determining the maturity index and % infestation (larvae + pupae + number of exit holes), the olives are divided into 3 lots: lot 1: healthy olives (which are not attacked by *B. oleae*) Called S; Lot 2: natural olives (reflecting the real attack rate of the fruit) called N, lot 3: only olives attacked (each olive has at least one exit hole) called A. In this work, only the olives were studied after their lyophilisation no oil extraction was performed.
The preparation of the olives for the various analysis was carried out in the Applied Biochemistry Laboratory. The olive powder was obtained by lyophilisation according to the following steps:
First, the olives were cut into thin pieces and frozen at (-80°C);
The second step consists of lyophilization at (-58°C);
Finally, grinding in an electric mixer was carried out and then the sample was stored at (-18°C) to preserve the composition of the olives.

2.6. PROFILE OF PHENOLIC COMPOUNDS BY HPLC

The solid-liquid extraction method was used for the extraction of phenolic compounds according to the method described by Mc Donald et al. [15]. The freeze-dried olives were macerated in MeOH-water, stirred then centrifuged. The pellet was recovered for a second extraction and the supernatant was washed in triplicate with hexane to remove all traces of lipid. The hydrophilic phase was recovered by decantation and then filtered.
The chromatographic analysis was carried out in an integrated HPLC system equipped with an LC-NetII / AD43, an AS-2057 automatic sampler, a PU-2089 PLUS pump, a CO-2060 PLUS thermostat column, a multi-wavelength diode Network detector MD-2018 (DAD) connected in series to a fluorescence detector FP-2020 PLUS (Jasco, Japan).
A Zorbax SB-C18 column (250 × 4.6 mm, 5 mm) from Agilent Technologies (Waldbronn, Germany) was used for the separation of the compounds, according to the conditions described [16], with some modifications.
A solvent gradient system consisting of acetic acid in water (5% v/v) (eluent A) and methanol (eluent B) was used as follows: 0': 15% B; 10': 28% B; 15': 28% B; 16': 30%; 40': 40% B; 45': 45% B; 60': 100% B. The elution is carried out at 30°C, using a flow rate of 1 mL / min, the injection volume being 20 µL. The chromatograms were recorded at 240 nm, 280, 320 and 335 nm, based on the maximum absorption wavelengths of each compound analysed [17]. In addition, hydroxytyrosol and tyrosol were followed by fluorescence (λexc: 280 nm, λem: 330 nm) [18]. The chromatographic data were analysed using PDA-Borwin Controller software (JMBS, France). The compounds were identified by chromatographic comparison with authentic standards and by their UV spectrum.

2.7. ANTIOXIDANT ACTIVITIES

2.7.1. Reducing power

The reducing power of the samples was determined [19]. Phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%) solutions were prepared and added to 1 mL of each sample. After

incubation during 20 min at 50°C, 2.5 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 1500 g for 10 min. An aliquot (2.5 mL) of the upper layer of the solution was mixed with 2.5 mL of ultrapure water and 0.5 mL of FeCl solution (0.1%). The absorbance of each mixture was measured at a wavelength of 700 nm. The increase in the absorbance values can be correlated with the reducing power that was expressed as mg caffeic acid equivalents (CAE) per 100 g of DM.

2.7.2. ABTS assay

The antioxidant activity of olive extracts was determined using a 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonicacid (ABTS) radical cation discoloration assay [20]. Succinctly, 3.9 mL of diluted ($ABTS^{+•}$) solution was added to 100 μL of a phenolic fraction or Trolox. The mixture absorbance was read at 734 nm, at 30°C, exactly after 6 min of the initial mixing.

2.7.3. Chelating capacity

The chelating capacity of the methanolic extracts of four Algerian olive cultivars was determined [21]. This method is based on the inhibition of the formation of the Fe (II)-Ferrosine complex after the treatment of the samples with the Fe^{2+} ions. Five hundred microliters of the extract solutions were added to 100 μL of FeCl (0.6 mM) and 900 μL of methanol. After 5 min of incubation, 100 microliters of ferrosine (5 mM) were added and the mixture was stirred and allowed to react for 10 min to allow the complexing of the residual iron. The absorbance of the ferrozine - Fe^{2+} complex was measured at 562 nm.

2.7.4. Relative antioxidant capacity index (RACI)

The results of the antioxidant activity obtained by the above-described chemical methods were integrated by calculating the Relative Antioxidant Capacity Index (RACI). The RACI index allows the comparison of antioxidant capacity derived from different chemical methods (Reducing power and Chelating capacity).
To calculate the relative index of the antioxidant capacity of each sample, we started by calculating the standard score according to the following formula:

$$scorestandrds = \frac{x - \mu}{\sigma}$$

Where:
X = is the raw data,
$\mu$is = the mean, and σ is the standard deviation [22].

2.8. STATISTICAL ANALYSIS

The statistical analysis was carried out using the software Statistica 5.5. For each parameter, the analysis of the variance (ANOVA) was used, followed by the Newman & Keuls test with a confidence level of 95% ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. FRUIT WEIGHT (FW), MATURITY INDEX (MI) AND ATTACK RATE (AR) OF OLIVE FRUITS

The fruit weight (FW), the attack rate (AR) and the maturity index (MI) of the unsorted olive samples of the four cultivars studied are summarised in Table I. It appears that the variety exerts a significant effect on fruit weight ($p \leq 0.05$). Rougette de Mitidja has the highest weight (2.81 g) while Souidi has the lowest one (0.97 g).
The size of the fruit has a significant influence on the susceptibility of olives to attacks by B. oleae. The lowest attack rate (21%) was recorded by the Souidi variety which has the smallest fruits (0.97 g). Conversely, heavy olives were the most attacked. A significant positive correlation ($r = 0.91$) was noted between attack and the weight of fruits. Our results agreed with those of several authors who found that the fly prefers large-fruited varieties for egg laying [23 - 25]. Also, a relationship was highlighted between olive size and the percentage of fly attack; the largest olives exhibited the highest infestation [26, 27, 11].
Cultivar and maturation were crucial aspects in the olive fly preference [28]. The susceptibility of 20 most widely distributed mill and table olive Spanish varieties was studied [29]. Even though the olive fruit fly damaged all varieties, significant differences in susceptibility were detected among the mill olive and among the table olive varieties. Even though the diameter and oil content were positively correlated with B. oleae fruit infestation (correlation coefficients ranged between 0.5 and 0.95), their work reveals that other yet-unknown factors may influence B. oleae oviposition preferences.
Some of the factors related to fruit traits that possibly play a role include fruit size and mass, colour, fruit exocarp hardness, surface covering (mainly of aliphatic waxes), phenological stage of the crop, and chemical composition of olive fruits [30]. Recently, it was reported that *B. oleae* adult females mainly rely on olfactory cues, namely volatile organic compounds

Table I - Fruit weight (FW), attack rate (AR) and maturity index (MI) of four Algerian olive cultivars.

	FW (g)	AR (%)	MI
Abani	1.61±0.1 (b)	34.67±0.94 (b)	5.74±0.012 (c)
Ferkani	2.35±0.05 (c)	44.67±6.85 (b)	3.34±0.097 (a)
Rougette de Mitidja	2.81±0.05 (d)	65.33±9 (c)	4.67±0.008 (b)
Souidi	0.97±0.02 (a)	21±0.82(a)	6.54±0.008 (d)

The mean within each column labeled by different letters indicate a significant difference ($P < 0.05$).

Table II - Individual and total phenolic compounds of four Algerian olive cultivars (mg/Kg DM).

Variété	State	HT	Tyr	Ole.	Ver	AC	L-7-G	Rut.	A-7-G	Total HPLC
Abani	S	103.91±1.52(de)	30.97±0.51(d)	41.48±1.48(a)	1265±15.63(g)	0.00	165.48 ± 2.51(ef)	263.97±0.33(d)	3.96±0.05(c)	1875.17±12.84(c)
	N	93.68±1.61(de)	24.15±1.09(c)	92.27±3.90(a)	1062.67±19.20(i)	0.00	157.94±9.38(ef)	259.04±1.48(d)	4.05±0.21(c)	1693.79±36.87(d)
	A	54.05±1.73(be)	21.99±0.44(bc)	60.62±1.37(a)	840.56±11.91(e)	00	151.32±2.67(e)	211.77±9.04(c)	3.84±0.10(c)	1344.95±26.38(b)
Ferkani	S	17.98±0.19(a)	16.45±0.99(ab)	3789.72±40.37(f)	1458.63±16.87(j)	0.00	171.58±6.13(f)	141.09±1.41 (b)	2.14±0.03(a)	5597.60±65.97(j)
	N	10.86±0.28(a)	12.26±0.20(a)	2626.96±104.46(e)	926.98±32.07(f)	0.00	147.84±2.71(e)	119.92±4.62 (b)	2.07±0.00(a)	3846.89±143.94(h)
	A	9.87±0.14(a)	13.07±0.09(a)	1721.39±49.49(d)	713.12±26.55(d)	0.00	109.79±0.49(c)	82.06±2.31(a)	2.00±0.01(a)	2651.30±79.07(f)
Rougette de Mitidja	S	374.89±14.74(f)	152.27±5.49(f)	1386.19±29.23(c)	2530.67±48.67(k)	0.00	130.45±5.50(d)	121.36±2.51 (b)	3.80±0.07(c)	4699.64±90.19(i)
	N	71.45±0.58(cd)	33.76±1.61(de)	1314.85±7.29(b)	1769.17±159.83(f)	0.00	92.55±5.27(bc)	132.48±7.15 (b)	3.56±0.20(c)	3417.83±153.47(e)
	A	55.2± 2.42(g)	37.72±1.63(e)	1086.14±47.97(c)	1185.09±7.28(h)	0.00	95.35±2.58(bc)	86.67±2.68(a)	4.62±0.20(d)	2550.79±31.18(g)
Souidi	S	112.36±3.72(d)	11.81±0.25(abc)	65.14±0.84(a)	478.59±1.86(c)	24.63±0.17(d)	62.09±0.60(a)	428.51±1.87(f)	2.06±0.05(a)	1185.21±6.39(b)
	N	72.70±0.30(cd)	19.66±0.44(c)	51.84±1.74(a)	393.26±10.00(b)	13.49±0.63(c)	102.86±3.73(bc)	444.74±13.21 (f)	2.26±0.05(a)	1100.81±28.15(b)
	A	33.39± 0.93(ab)	13.19±0.25(a)	24.69±0.02(a)	166.14±3.64(a)	11.83±0.22(b)	85.22±2.14(b)	341.62±7.88 (e)	2.88±0.02(b)	678.95±14.54(a)

The mean within each column labeled by different letters indicate a significant difference ($P < 0.05$). State of olives – healthy olives (S), natural (reflecting the real attack rate of the fruit) (N), only attacked olives (A).
Phenolic compounds: AC, cafeic acid; HT hydroxytyrosol; Tyr: tyrosol; OLE: oleuropein;Ver, verbascosid; Rut, rutin; L-7-G, Luteolin-7-glucoside; A-7-G, apigenin-7-glucoside;

produced by the tree [31]. Correlation between infestation level during olive maturation and the aromatic hydrocarbon toluene from olive leaves from different cultivars had been observed previously [9].

3.2. PHENOLIC COMPOSITION

The chromatographic analysis of olives phenolic extracts showed a qualitative composition of phenolic compounds almost similar for all the samples, but different from a quantitative point of view. Eight compounds hydroxytyrosol, tyrosol, oleuropein, verbascosid, caffeic acid, luteolin, rutin and apigenin were identified (Table II).

By comparing total polyphenol levels, for all studied varieties, *Ferkani* had the highest grade (5597.6 mg EAG / kg) and *Souidi* had the lowest (1185.21 mg EAG / kg). A significant difference ($p = 0.05$) was noted among cultivars. It is important to note that the different varieties studied didn't have the same degree of maturity (it is 3.34 for *Ferkani* cultivar and 6.54 for the *Souidi* cultivar). A negative correlation was noted between maturity and phenolic content ($r = -0.99$). The polyphenol content decreases progressively during the maturation process [32], this decrease can reach 30% depending on the variety [33]. The values obtained in this study were far inferior to those found by Ben Othman et al. [34] which is 17600 mg / kg MS.

The total polyphenol losses are maximal in the 100% attacked sample from *Ferkani* cultivar, it was 52.64% followed by the *Souidi* cultivar 42.71%. In the two remaining cultivars *Rougette de Mitidja* and *Abani*, the respective losses were 30, 28%.

The maximum losses recorded in our study are much higher than those found by Koprivnjak et al. [35], which are 21% in the Istarska variety from Croatia, and lower than the value found in the Chemlali variety from Tunisia of 83% [36]. This is due to the specific phenolic profile of olives, which depends on the variety [37]. This difference is due to, according to Koprivnjak *et al.* [35], fruit properties that are not conducive to larval development, and low volume of the mesocarp of infested fruit, which is the reason for the low degradation of polyphenols.

The two phenolic alcohols; hydroxityrosol (3, 4-DHPEA) and tyrosol (p-HPEA) have the highest levels in the *Rougette de Mitidja* cultivar (representing 19.37% and 6.33% of the total polyphenol contents respectively). They decrease drastically with the infestation level. A significant negative correlation was noted between the attack and these two compounds. The *Rougette de Mitidja* variety was the most affected by the attack and had the highest loss rates of 85.88% and 75.05% for hydroxytyrosol and tyrosol respectively, followed by of *Abani* cultivar (47.98% and 29% respectively).

Oleuropein varied quantitatively from one variety to another, ranging from 3.6% to 67.7% of the total phenols for the *Abani* and *Ferkani* cultivars respectively.

Ferkani cultivar has an oleuropein content two times higher than the *Rougette de Mitidja* cultivar despite the cultivars have almost the same maturity index and *Abani* and *Souidi* cultivars with advanced maturity stages have the lowest levels. In general, cultivars with a small-size fruit have higher concentration of oleuropein compared to large-sized fruit cultivars during developmental stages [38]. On his part, Bianchi, reported that at the beginning of ripening, oleuropein was the most abundant compound in olives and its concentration reached up to 14% of the dry matter of young fruit [39]. This decrease of oleuropein during maturation inversely was correlated with the increase in oleuropein derivatives, especially hydroxytyrosol. The losses, which vary according to the cultivar, were very pronounced in the Souidi (62.1%) and Ferkani (54.58%) cultivars, while the rest of the varieties recorded values below 50%.

According to Spadafora et al. [40], the defence molecules in olives were phenols synthesised and accumulated in fruit tissues during growth and maturation. The main defence component among these phenols was the phenolic β-glucoside secoiridoid, oleuropein, a bitter molecule characteristic of olives. This compound possessing antioxidant and antimicrobial activity has been referred to as a defence molecule against insect attack. When the olive tissues are injured by pathogens or by mechanical damage, β-glucosidase, belonging to the family of glucohydrolase enzymes, specifically hydrolysing oleuropein to produce highly reactive molecules. The olives contain large amounts of β-glucosidase, which specifically hydrolyses oleuropein. Gucci et al. [41], claimed that the main phenolic compounds affected by olive fly infestation were the secoiridoids. Gomez-Caravaca et al. [42], reported significant losses of simple phenols, lignans and secoiridoids.

All the varieties studied showed appreciable levels of verbascosid. *Rougette de Mitidja* cultivar was characterized by the highest content 2530.67 mg/kg, followed by *Ferkani* and *Abani* cultivars with respective contents of 1458.63 and 1062.67 mg/kg. The lowest value was found for the *Souidi* cultivar (478.59 mg/kg). Substantial losses were recorded and up to 65.29% in the *Souidi* cultivar. The two cultivars *Rougette de Mitidja* and *Ferkani* also have high loss rates, which were 53.17% and 51.11%, respectively. As for *Abani* cultivar, it showed only a loss of 20.90%.

Caffeic acid was present in trace amounts in most of the studied cultivars, except for the *Souidi* cultivar, which had 24.63 mg / kg. The most important loss of caffeic acid was recorded with the *Souidi* variety, which was 51.97% for the sample attacked 100%.

Three flavonoids were determined in the four analysed olive cultivars: luteolin (L7G), rutin and apigenin (A7G). Luteolin quantitatively occupied the second position of the total flavonoid content after rutin. *Ferkani* had the highest content (171.58 mg/kg) followed by the *Abani* and *Rougette de Mitidja* cultivars with respective grades of 165.48 mg/kg and 130.45 mg/kg. Concerning *Souidi* cultivar, it had only an amount of 62.09 mg/kg.

Rutin was the main flavonoid in the analysed olive varieties. The maximum level was recorded in the *Souidi* variety (428.51 mg/kg) followed by the *Abani* cultivar (263.97 mg/kg). However, the two remaining varieties, *Ferkani* and *Rougette de Mitidja*, showed only 141.09 and 121.36 mg/kg respectively. These results lead us to conclude that rutin is present in larger quantities in small olive varieties. Significant losses were reported of rutin for some olives varieties up to 41.84% for *Ferkani*. For *Abani*, *Rougette de Mitidja* and *Souidi* cultivars, the respective losses were: 19.77%, 28.58% and 20.28%.

Apigenin, the minor flavonoid of the analysed olives cultivars, was identified at very low levels. The studied

Table III - Antioxidant activities of four Algerian olive cultivars

Variety	State	Reducing power (mg CAE / 100 g of DM)	ABTS assay (%)	Chelating capacity (mg EEDTA/100g DM)
Abani	S	206.42±1.79(f)	70.14±10(f)	32.51±0.12(ef)
	N	149.90±6.87(e)	63±1.82(de)	28.43±0.43(cd)
	A	66.57±6.46(b)	56.19±0.89(c)	22.29±0.05(ef)
Ferkani	S	359.67±11.15(h)	56.24±2.36(c)	32.72±1 .74(ef)
	N	238.51±6.21(g)	48.24±2.75(b)	26.76±0.24(bc)
	A	80.94±4.88(c)	40.76±1.50(a)	22.72±0.09(a)
Rougette de Mitidja	S	680.56±2.95(i)	62.86±0.71(de)	41.8±0.07(h)
	N	136.97±4.12(e)	46.33±0.47(b)	36.71±2.88(g)
	A	94.83±3.1(d)	38±0.61(a)	29.89±0.05(de)
Souidi	S	146.55±3.10(e)	65.24±0.99(e)	35.01±0.2(fg)
	N	57.95±3.77(b)	60.52±0.95(d)	29.92±1.49(de)
	A	45.02±2.95(a)	53.67±1.75(c)	24.83±0.55(ab)

The mean within each column labeled by different letters indicate a significant difference ($P < 0.05$). State of olives – healthy olives (S), natural (reflecting the real attack rate of the fruit) (N), only attacked olives (A).

Table IV - Correlation matrix between phenolic compounds and antioxidant activity.

	VAR	A	MI	HT	Tyr	Ole	Ver	AC	L-7-G	Rut	A-7-G	TP	RP	ABTS	CC
VAR	1,000														
A	0,613*	1,000													
MI	0,106	-0,610*	1,000												
HT	0,623*	-0,175	0,820*	1,000											
Tyr	0,948*	0,528	0,271	0,674*	1,000										
Ole	-0,328	0,465	-0,966*	-0,937*	-0,441	1,000									
Ver	0,538	0,454	-0,379	0,139	0,261	0,152	1,000								
AC	-0,471	-0,688*	0,704*	0,197	-0,207	-0,503	-0,907*	1,000							
L-7-G	-0,290	-0,233	-0,270	-0,188	-0,526	0,217	0,606*	-0,457	1,000						
Rut	-0,330	-0,807*	0,898*	0,489	-0,127	-0,759*	-0,650*	0,905*	0,207	1,000					
A-7-G	0,869*	0,318	0,247	0,746*	0,753*	-0,481	0,723*	-0,491	0,139	-0,179	1,000				
TP	-0,200	0,511	-0,986*	-0,838*	-0,382	0,963*	0,407	-0,695*	0,406	-0,853*	-0,267	1,000			
RP	-0,152	0,427	-0,916*	-0,709*	-0,395	0,854	0,591	-0,790*	0,612*	-0,835*	-0,103	0,956*	1,000		
ABTS	-0,152	-0,775*	0,790*	0,622*	-0,125	-0,776*	-0,079	0,459	0,323	0,767*	0,233	-0,705*	0,526	1,000	
CC	0,720*	0,641*	0,088	0,346	0,850*	-0,176	-0,007	-0,082	0,705*	-0,161	0,392	-0,210	0,335	0,395	1,000

* Signifiant at P<0.05.
Var: variety, A: attack; MI: maturity index; HT: hydroxytyrosol; Tyr: tyrosol; OLE: oleuropein; Ver: verbascosid; AC: cafeic acid; HT: hydroxytyrosol; Tyr: tyrosol; OLE: oleuropein; Ver: verbascosid; Rut: rutin; L-7-G: Luteolin-7-glucoside; A-7-G: apigenin-7-glucoside; Rut: rutin; L-7-G: Luteolin-7-glucoside; A-7-G: apigenin-7-glucoside; TP: total phenol; RP: reducing power; CC: chelating capacity

cultivars have only very low levels, ranging from 2.06 to 3.96 mg/kg for *Souidi* and *Abani* cultivars respectively. The losses, in all the analysed cultivars, do not exceed 6.5% (noted for the *Ferkani* cultivars).
The main reasons for the loss of biophenols according to Koprivnjak et al. [35] are most likely an increase in endogenous polyphenoloxidase activity due to the damage of the cellular structure and the exposure to oxygen due to exit holes on the surface of the fruit. The changes induced by the attack of the olive fly on the expression of some key genes in the biosynthesis of volatile and phenolic compounds, such as lipoxygenase, beta-glucosidase, and polyphenol oxidase, have been analysed in olives of three cultivars (Picual, Manzanilla, and Hojiblanca) [43]. The results showed a strong induction of a new olive polyphenol oxidase gene (oeppo2) which explains the reduction of phenolic content in the oils obtained from infested fruits and suggest the existence of a PPO-mediated oxidative defence system in olives.

3.3. ANTIOXIDANT ACTIVITIES

The results of the antioxidant activity of the studied olive samples measured by three different methods are summarised in Table III.

3.3.1. Reducing power

The capacity of the olive samples to reduce Fe^{3+} to Fe^{2+} varied widely according to the fly attack degree. Values found in this study (Table III) decreased in attacked samples of all cultivars (from 680.56 to 94.83 mg CAE per 100 g of DM for *Rougette de Mitidja*, from 359.67 to 80.94 mg CAE per 100 g of DM for *Ferkani* from 206.42 to 66.57 mg CAE per 100 g of Dry Matter (DM) for *Abani*, and, finally, from 146.55 to 45.02mg CAE per 100 g of DM for *Souidi* cultivar). Losses of activity were about 86%, 77%, 69 and 67% for *Rougette de Mitidja*, *Ferkani*, *Souidi* and *Abani* respectively. *Rougette de Mitidja* being more susceptible to a fly attack. The *B. oleae* attack influences significantly the reducing power values of olives. The drastically decrease of the reducing power activity of olive from the attacked samples was due to the decrease in antioxidants (positive correlation was noted between reducing power and phenolics, $r = 0.96$), used probably to protect the fruit against the action of *B. oleae* larvae.

3.3.2. ABTS assay

Phenolic extracts of four studied cultivars showed a high scavenging capacity estimated according to the ABTS-RSC assay. The values determined (Table III) showed a decreasing tendency in the olives (from 70.14 to 56.19%, from 56.24 to 40.76%, from 62.86 to 38% and from 65.24 to 53.67% for *Abani*, *Ferkani*, *Rougette de Mitidja* and *Souidi* cultivars respectively). The changes verified can be justified by the decreasing levels of available antioxidants, as explained previously. It is noteworthy that activity losses estima-

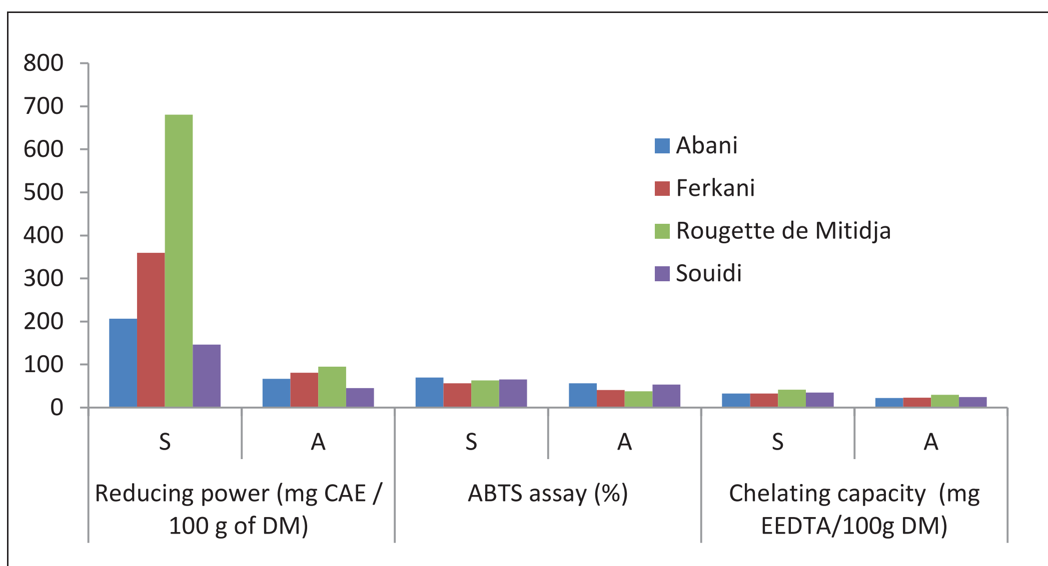

Figure 1 - Antioxidant activities of healthy and infested samples of studied cultivars.
S: Healthy olives; A: attacked olives

ted by ABTS-RSC assay in attacked samples were 19.89%, 27.27%, 39.55% and 17.73% in *Abani*, *Ferkani*, *Rougette de Mitidja* and *Souidi* cultivars respectively. The determined ABTS values exhibited a similar behaviour described for the reducing power. Medjkouh et al. [44] have found same results in another study on the antioxidant activity of olive oils from olives attacked by *B. Oleae*.

3.3.3. Chelating capacity

Phenolic extracts of studied olive cultivars showed an important chelating capacity. Olives exhibited almost the same activity, which was from 32.51, 32.72, 41.8, 35.01 mg EDTA/100 g DM for *Abani*, *Ferkani*, *Rougette de Mitidja* and *Souidi* cultivars respectively. Losses recorded in this study were very close and ranged from 28.49%, 29.08%, 30.56% and 31.44% for *Rougette de Mitidja*, *Souidi*, *Ferkani* and *Abani* respectively.

Antioxidant activity decreased drastically in samples infested by the olive fruit fly comparatively to the healthy samples as showed in Figure 1. This is due to the significant losses of antioxidants in the olive fruits. Janji et al. [45] reported in their work the effect of infestation by the olive fruit fly *Bactrocera oleae Gmel* on the stability of olive oil. The latter recorded a clear decrease following the great losses of polyphenols, tocopherols, and pigments (chlorophyll and carotenoids).

3.4. RELATIVE ANTIOXIDANT CAPACITY INDEX (RACI)

The values of the Relative Antioxidant Capacity Index (RACI), which represent the average scores of the antioxidant activities of each sample, were shown in Figure 2.

The RACI was validated as a reference for ranking samples according to their antioxidant potential which

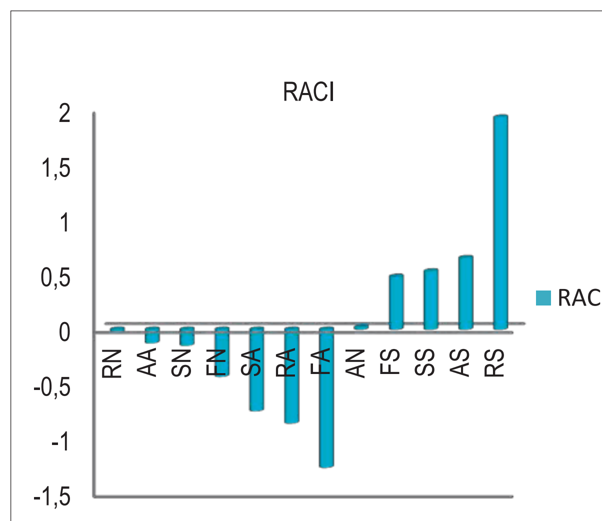

Figure 2 - Relative Antioxidant Capacity Index (RACI) of olive fruits samples. Cultivars (first letter) – Abani (A); Ferkani (F), Rougette de Mitidja (R), Souidi (S); State of olives (second letter) – healthy olives (S), natural (reflecting the real attack rate of the fruit) (N), only attacked olives (A).

results from the combination of all the methods used, because it makes the comparison of the data which should follow a normal distribution more reliable.

From this figure we can affirm that the extract of healthy olives of the *Rougette de Mitidja* cultivar marked the superiority in its contribution to all the tests, mentioning an RACI of +1.993. The lowest RACI value was recorded by the extract of attacked olives of the *Ferkani*, attacked variety (-1.262).

The order of classification can be given as follows: [(*Ferkani*, attacked), -1.262] < [(*Rougette de Métidja*, attacked), -0.858] < [(*Souidi*, attacked), -0.745] < [(*Ferkani*, natural), -0.430] < [(*Souidi*, natural), -0.145] < [(*Abani*, attacked), -0.123] <[(*Rougette de Métidja*,

natural), -0.036] < [(*Abani*, natural), +0.02] < [(*Ferkani*, healthy), +0.477] < [(*Souidi*, healthy), +0.526] < [(*Abani*, healthy), +0.647] < [(*Rougette de Métidja*, healthy), +1.930].

Most of the positive RACI values were recorded by the healthy samples for the four varieties. Among the natural samples, only the Abani variety showed a very small positive value (0.020). This can be elucidated by the diversity of their phenolic compounds, which differ in their quantities and relativities.

4. CONCLUSION

The current work yielded information on the olive fruit fly on antioxidants and the antioxidant activity of four olive varieties grown in Algeria.

Antioxidant potential was reduced due to the loss of antioxidant compounds, as it is the case of phenolic compounds, namely hydroxytyrosol, tyrosol and oleuropein, as already witnessed and reported in this study. Olives with an infestation higher than 20% have a loss rate between 30% and 52%.

Regarding olive pests and diseases, olives are primarily affected on the economic field since significant losses are entailed each year in the olive fruit production. The quality and composition of olive oils are significantly modified by the olive fly. The actions of olive flies are so serious that olive oils are often downgraded and this has a negative impact on the international market.

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