

# Variation in olive phenolics and antioxidant activity: influence of variety, location, and *Bactrocera oleae* attack

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## Abstract

The olive tree (*Olea europaea* L.) holds significant economic importance, especially in the Mediterranean region, including Algeria, where diverse olive cultivars thrive due to the heterogeneous climate. This study presents a comparative analysis of phenolic and antioxidant properties in olive fruits from two Algerian cultivars, 'Chemlal' and 'Sigoise', focusing on the influence of different locations and varying attack rates of the olive fruit fly (*Bactrocera oleae*). Key biological parameters, including fruit weight, maturity index, and pest attack rates, alongside the content of phenolics, flavonoids, and condensed tannins were measured. Antioxidant activity was evaluated by measuring free radical scavenging, total antioxidant capacity, and ferric-reducing power. Significant variation in fruit characteristics and insect susceptibility between the two cultivars and locations was found. Cv. 'Sigoise' exhibited greater fruit weight but higher vulnerability to *Bactrocera oleae* attacks than cv. 'Chemlal'. Fruits of cv. 'Chemlal' from Ain Arnat showed greater insect attack resistance and higher condensed tannin content. Antioxidant assays revealed that cv. 'Chemlal', especially from Ain Azel, had superior free radical scavenging and ferric-reducing ability, despite its lower phenolics content, indicating a robust antioxidant profile. This study demonstrates the potential of selecting and cultivating specific olive cultivars to optimize their health-promoting benefits and resistance to biotic stress. This underlines the need for tailored agronomic practices that consider genetic, environmental, and pest management factors for enhanced productivity and quality.

**Key words:** antioxidant properties, *Bactrocera oleae*, environmental factors, *Olea europaea* L., olive cultivars, phenolic content.

**Abbreviations:** DE, dry extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC<sub>50</sub>, half maximal effective concentration; EAA, ascorbic acid equivalent; GE, gallic acid equivalent; IC<sub>50</sub>, half-maximal inhibitory concentration; QE, quercetine equivalent; TAC, total antioxidant capacity; TC, tannin content; TFC, total flavonoid content; TPC, total polyphenol content.

## Introduction

*Olea europaea* L., commonly known as the olive tree, holds great importance within the Oleaceae family. Its fruits and oil are crucial to the economies of many countries and regions worldwide. Due to heterogeneous climate, Algeria boasts diverse ecosystems and is recognized as a secondary diversification centre for various olive subspecies and cultivars. Several olive cultivars have been identified, and a high degree of morphological and biological variation exists (Djelloul et al. 2020; Atrouz et al. 2021; Issaad et al. 2024).

The main qualitative characteristics of olive fruits include fruit weight, oil content, phenolic profile, and fatty acid composition (Cheng et al. 2017). Olive fruit is rich in phenolic compounds varying from 1 to 3% of fresh pulp weight. Primary compounds identified in olive

include phenolic acids, phenolic alcohols, flavonoids, and secoiridoids, the latter being exclusively present in the Oleaceae family (Dekdouk et al. 2015). Polyphenol content in fruit has been linked with several health benefits, and these compounds are characterized by the presence of conjugated double bonds, hydroxyl, and carboxyl groups (Calderón-Oliver, Ponce-Alquicira 2018), that enable them to act as antioxidants, affecting the oxidation process through various mechanisms, including prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of further hydrogen abstraction, and radical scavenging. The rich antioxidant profile of olive fruits makes them a valuable component of a healthy diet, contributing to the prevention of various oxidative stress-related diseases such as diabetes, cardiovascular and inflammatory diseases, cancer, and metabolic syndrome (Tekeshwar, Vishal 2016; Calderón-

Oliver, Ponce-Alquicira 2018).

The biosynthesis of biochemical components in plants is shaped by multiple factors, with genetics playing a pivotal role (Francini et al. 2020). The phenolic profiles of different olive cultivars across regions vary significantly due to genetic factors. This genetic variability affects the formation and concentrations of oleuropein, hydroxytyrosol, lignans, and flavonoids, and furthermore, it influences the levels of specific phenolics and plays a key role in determining antioxidant activity and flavour profiles of olive fruits (Servili et al. 2004; Matos et al. 2007). Environmental conditions can positively or negatively alter the concentration of bioactive compounds in horticultural crops (Cheng et al. 2017). Studies on Italian and Turkish olive cultivars demonstrate this impact on phenolic content and antioxidant properties. Italian olives from higher altitudes exhibited greater antioxidant properties due to environmental stress (Rochetti et al. 2020), while geographic origin in Turkish olives affected antioxidant capacity and phenolic content, influenced by factors like soil and water availability (Ozturk et al. 2021). In addition to environmental factors, biotic stressors also play a critical role. The olive fruit fly (*Bactrocera oleae*), one of the most significant biotic threats to olive fruits, causing damage by laying eggs beneath the fruit's skin (Valenčič et al. 2021). Upon hatching, larvae feed on the mesocarp, leading to mechanical destruction of plant tissues. Oviposition also opens the way for secondary infestations of bacteria and fungi, which contribute to fruit rot and degrade the quality of the olive (Valenčič et al. 2021). Infestations by the fly have been shown to reduce phenolic content and antioxidant properties in olives, as pest damage leads to oxidative degradation and declines in key compounds like oleuropein and verbascoside, which are crucial for antioxidant activity (Medjkouh et al. 2016).

These recent studies collectively highlight that a combination of genetic, environmental, and biotic factors determines the phenolic and antioxidant profiles in olive fruits. The present study employs a comparative approach to evaluate the phenolic and antioxidant properties of olive fruits from two Algerian cultivars, 'Chemlal' and 'Sigoise', collected from two distinct locations in Setif. It was examined how these properties correlate with fruit weight, maturity levels, location, and attack rate by *B. oleae*. The paper provides an analysis of how these factors vary across different environmental conditions.

## Materials and methods

### Sampling and biological characterization

Both 'Sigoise' and 'Chemlal' are prominent cultivars in Algeria and are widely cultivated, making them highly relevant for studies on local agricultural practices and improving olive oil quality. Olive fruits from two Algerian cultivars, 'Sigoise' and 'Chemlal', were harvested from two

olive groves in two locations: Ain Azel (35.800915°N, 5.508044°E) and Ain Arnat (36.154934°N, 5177631°E), resulting in four olive groups (C1, 'Chemlal' from Ain Azel; C2, 'Chemlal' from Ain Arnat; S1, 'Sigoise' from Ain Azel; S2, and 'Sigoise' from Ain Arnat). Ain Arnat and Ain Azel, in Algeria's Sétif province, have different environmental characteristics due to their locations within three climatic zones. Ain Azel, in the southern part, is semi-arid, with summer temperatures averaging around 30 °C and low annual rainfall, which limits traditional crop farming and requires drought-resistant practices (Bougherra, Chehat 2018; Bouziane et al. 2021). In contrast, Ain Arnat, located centrally and at a slightly higher elevation, enjoys cooler temperatures and higher rainfall, allowing for a wider range of rainfed crops (Bougherra, Chehat 2018; Kouidri, Sahli 2020).

Fruit samples were collected from ten trees of each cultivar, with fruits (eight to ten olives) taken from the four cardinal directions around the tree. The maturity index (MI) of olive fruits was calculated based on the method described by the International Olive Council (2011), using the following formula to quantify the stages of ripening in olive:

$$MI = A0 + B1 + C2 + D3 + E4 + F5 + G6 + H7 / 100.$$

Olives were categorized into eight ripening stages based on skin and flesh color, ranging from deep green (Category 0) to black with all flesh purple to the stone (Category 7). The number of fruits in each category was counted and recorded as A through H, representing Categories 0 to 7, respectively. Higher MI values indicate more advanced ripening stages in the sample.

The weight of the fruit from the studied cultivars was measured by weighing 100 randomly selected drupes from the samples (Mahhou 2014). The attack rate of *B. oleae* was estimated using the formula by Kaul et al. (2009), based on the number of attacked olives (larvae + pupae + exit holes) in a randomly sampled batch of 100 olives taken after harvesting.

The fruit's pulp and skin was separated from the stone, and then shade-dried at room temperature, mechanically ground, and stored at 4 °C until analysis.

### Extract preparation

Extracts of olive were prepared as follows: 5 g of dehydrated olives were macerated in 100 mL ethanol (70%) for five days (4 °C). The mixture was filtrated, and the solvent was removed using a rotary evaporator. The residue was then dried in an oven (40 °C) until constant weight was achieved. The resulting dry extract was stored at 4 °C (Krishna et al. 2019).

### Total polyphenol content determination

The total polyphenol content (TPC) of extracts was determined using the Folin-Ciocalteu method (Karbab et al. 2020a). In short, 500 µL of Folin-Ciocalteu reagent was

mixed with 100  $\mu\text{L}$  of olive extract dissolved in distilled water for 4 min before adding 400  $\mu\text{L}$  of  $\text{Na}_2\text{CO}_3$  aqueous solution (7.5%). The absorbance of the resulting solution was measured at 765 nm after 2 h of incubation. The polyphenolic content was quantified and expressed as  $\mu\text{g}$  of gallic acid equivalent (GE)  $\text{mg}^{-1}$  of dry extract (DE).

#### Total flavonoid content determination

The total flavonoid content (TFC) of the samples was assessed using the aluminum chloride method: 1 mL of  $\text{AlCl}_3$  methanolic solution (2%), was added to 1 mL of the extracts solubilized in methanol (90%). After a 10-min incubation period, absorbance at 430 nm was measured. Quercetin served as the standard, and the results were expressed as  $\mu\text{g}$  of quercetin equivalents (QE)  $\text{mg}^{-1}$  DE (Karbab et al. 2021).

#### Condensed tannin content determination

A 50  $\mu\text{L}$  volume of each extract, dissolved in methanol 90%, was added to 1500  $\mu\text{L}$  of a 4% vanillin/methanol solution and mixed thoroughly. Subsequently, 750  $\mu\text{L}$  of concentrated HCl was added to the mixture. The resulting solution was allowed to react at room temperature for 20 min. Absorbance was then measured at 550 nm against a blank solution reference. The condensed tannins content was expressed as  $\mu\text{g}$  catechin equivalents (CE)  $\text{mg}^{-1}$  DE (Amari et al. 2023).

#### Free radical scavenging assay

The free radical scavenging capacity of the extracts was evaluated using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay. Briefly, 50  $\mu\text{L}$  of various amounts (0.15625, 0.3125, 0.625, 1.25, 2.5, 5, 10  $\text{mg mL}^{-1}$ ) of the extract were mixed with 1.25 mL of DPPH methanolic solution (0.004%). The absorbance of the sample was then measured at 517 nm after incubation for 30 min in the dark at room temperature. Butylated hydroxytoluene was used as a positive control (Karbab et al. 2020).

The scavenging capacity was determined using the following equation:

$$I (\%) = (A^{\text{blank}} - A^{\text{test}}) / A^{\text{blank}} \times 100,$$

where  $A^{\text{blank}}$  is the absorbance of the solution excluding the tested sample and  $A^{\text{test}}$  is the absorbance of the tested sample.

#### Total antioxidant capacity assay

The total antioxidant capacity (TAC) in extracts was assessed using the phosphomolybdate method (Abhishek et al. 2013; Pavithra, Banu 2017). A 0.1 mL aliquot of various amounts (0.15625, 0.3125, 0.625, 1.25, 2.5, 5, 10  $\text{mg mL}^{-1}$ ) of extract was added to 1 mL of a reagent solution containing 600 mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate in a 1:1:1 ratio. The test tubes were then covered with aluminum foil and incubated in a water bath at 95 °C for 90 min. After cooling to room temperature,

the absorbance was measured at 695 nm. Ascorbic acid was used as a standard. The TAC was expressed as milligrams equivalent of ascorbic acid (EAA)  $\text{g}^{-1}$ .

#### Ferric reducing power assay

The capacity of the extract to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  ions was evaluated (Karbab et al. 2019). In this procedure, 400  $\mu\text{L}$  of extract (0.15625, 0.3125, 0.625, 1.25, 2.5, 5, 10  $\text{mg mL}^{-1}$ ) was combined with 400  $\mu\text{L}$  of 200 mM phosphate buffer (pH 6.6) and 400  $\mu\text{L}$  of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50 °C for 20 min. To stop the reaction, 400  $\mu\text{L}$  of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 min. After centrifugation, 400  $\mu\text{L}$  of the supernatant was mixed with 400  $\mu\text{L}$  of distilled water and 80  $\mu\text{L}$  of 0.1% ferric chloride solution. The absorbance of the resulting solution was measured at 700 nm after a 10-min incubation. A higher absorbance indicates a stronger reducing power of the extract.

#### Statistical analysis

Statistical analysis was performed using SPSS software (version 26). Tukey's post-hoc test independent t-tests, one-way ANOVA, and correlation analysis were applied to identify significant differences across all studied parameters. A significance level of  $p \leq 0.05$  was used.

## Results

#### Biological parameters

No significant difference in fruit maturity index between cvs. 'Chemlal' and 'Sigoise' was evident (Table 1). C2 had the highest maturity index, indicating a more advanced stage of ripeness, while C1 had the lowest. Additionally, results showed that the 'Chemlal' and 'Sigoise' cultivars matured differently between Ain Azel and Ain Arnat, with location playing a significant role in maturity differences.

The weight of fruits was slightly larger in C2 than C1 locations, and in S2 compared to S1 (Table 1). Both cultivars showed a non-significant larger weight when cultivated in Ain Arnat as compared to Ain Azel. Notably, fruits of cv. 'Sigoise' exceeded the weight of cv. 'Chemlal' fruits at both locations with a clear significant difference, indicating a cultivar-specific characteristic.

Unlike the other samples, C2 fruits exhibited the lowest attack rate by *B. oleae* (Table 1). In contrast, C1 and S1 showed moderate attack rates, while S2 had the highest, exceeding the other samples. A notable difference between the two varieties was observed, with fruits of cv. 'Sigoise' displaying a significantly higher attack rate compared to cv. 'Chemlal'.

#### Biochemical parameters

Fruits of C1 had lower phenolic content compared to that of S1 (Table 1). The values for C2 and S2 were quite close,

**Table 1.** Maturity index, fruit weight, attack rate, phenolic, flavonoid, tannin content of 'Chemlal' and 'Sigoise' olive fruit extract. Each reported value is the mean  $\pm$  SD of three replicates. Means in the same column followed by different letters are significantly different ( $p < 0.05$ ). DE, dry extract; GAE, gallic acid; QE, quercetin; CA, catechine

Samples	'Chemlal' in Ain Azel	'Chemlal' in Ain Arnat	'Sigoise' in Ain Azel	'Sigoise' in Ain Arnat
Maturity index	2.41 $\pm$ 0.59 a	5.24 $\pm$ 0.80 b	3.67 $\pm$ 0.30 a	4.26 $\pm$ 0.04 b
Weight (g)	1.48 $\pm$ 0.04 a	1.84 $\pm$ 0.05 a	2.68 $\pm$ 0.08 b	2.92 $\pm$ 0.03 b
Attack rate (%)	36.4 $\pm$ 0.4 ab	26.1 $\pm$ 0.4 a	35.0 $\pm$ 0.5 a	50.8 $\pm$ 0.7 b
Total phenolics (mg GAE 100 g <sup>-1</sup> DE)	888.5 $\pm$ 0.4 a	1004.8 $\pm$ 1.4 b	1274.7 $\pm$ 2.8 c	1033.6 $\pm$ 2.4 b
Total flavonoids (mg QE 100 g <sup>-1</sup> DE)	51.1 $\pm$ 0.1 a	48.7 $\pm$ 0.1 a	56.0 $\pm$ 0.2 b	55.6 $\pm$ 0.1 b
Total condensed tannins (mg CA 100 g <sup>-1</sup> DE)	70.8 $\pm$ 0.23 a	155.1 $\pm$ 1.4 b	127.3 $\pm$ 1.32 b	110.7 $\pm$ 1.4 ab

with S2 showing a slightly higher phenolic content. Overall, results indicated significant differences in TPC between cultivars, with 'Sigoise' tending to have higher TPC than 'Chemlal', particularly in Ain Azel.

There were no significant difference between cultivars regarding TFC. Samples showed minimal differences, with C1 and C2 being relatively similar (Table 1). S1 and S2 also showed minimal difference of TFC values. However, there were significant differences between locations, with fruits at Ain Azel exhibiting higher flavonoid content.

C1 had the lowest condensed tannin (CT) content, while C2 had the highest, revealing a significant increase when cultivated in Ain Arnat (Table 1). In contrast, S1 and S2 exhibited a decrease in CT content for cv. 'Sigoise' when grown in Ain Arnat. No significant differences within varieties or between locations were evident. However, one-way ANOVA revealed significant differences among the four samples, with C2 showing significantly higher CT levels.

The results presented in Table 2 show antioxidant activity of the four samples assessed through three different assays: DPPH scavenging activity, ferric reducing power (FRP), and total antioxidant capacity (TAC). Each assay provides unique information about the antioxidant potential of the samples, which is critical for understanding their overall efficacy in neutralizing free radicals.

All four extracts showed considerable scavenging activity in comparison with the positive control butylated hydroxytoluene. Fruit extracts of cv. 'Chemlal', especially

C1, showed the lowest IC<sub>50</sub> value, thus the highest DPPH scavenging activity. T-tests showed no significant differences between cv. 'Chemlal' and cv. 'Sigoise', or between locations.

FRP also varied across the samples, with C1 showing the lowest EC<sub>50</sub> value, indicating the highest ferric-reducing ability. S2 had a moderate reducing power, not significantly different from C2, but significantly lower than C1, while S1 demonstrated the highest EC<sub>50</sub> value, suggesting the lowest ferric reducing power among the tested samples. Significant differences between locations was evident, with fruits from Ain Azel showing higher antioxidant power.

The effectiveness of olive extracts in reducing Mo(VI) to Mo(V) varied significantly across different varieties. The total antioxidant capacity (TAC) values ranged from 15.5  $\mu\text{g mL}^{-1}$  for ascorbic acid to 1970  $\mu\text{g mL}^{-1}$  for S1. Ascorbic acid, a well-established antioxidant, exhibited the lowest EC<sub>50</sub> value, highlighting its superior antioxidant capacity compared to the olive extracts. Among the olive samples, C1 demonstrated the lowest EC<sub>50</sub> value (101.6  $\mu\text{g mL}^{-1}$ ), indicating the highest antioxidant capacity. C2 also exhibited relatively high antioxidant capacity with an EC<sub>50</sub> value of 148.7  $\mu\text{g mL}^{-1}$ , though this was significantly lower than that of C1. In contrast, S1 and S2 displayed higher EC<sub>50</sub> values (1970 and 1331  $\mu\text{g mL}^{-1}$ , respectively), indicating lower antioxidant capacities compared to C1 and C2. These results reveal a strong significant difference between cultivars.

**Table 2.** Antioxidant capacity of olive fruit extracts. Means in the same column followed by different letters are significantly different ( $p < 0.05$ ). IC<sub>50</sub>, a concentration that is efficient and achieves 50% DPPH radical scavenging activity; EC<sub>50</sub>, the concentration at which the absorbance is effectively 0.5

Samples	DPPH assay (IC <sub>50</sub> $\mu\text{g mL}^{-1}$ )	Ferric reducing power (EC <sub>50</sub> $\mu\text{g mL}^{-1}$ )	Total antioxidant capacity (EC <sub>50</sub> $\mu\text{g mL}^{-1}$ )
'Chemlal' in Ain Azel	46.530 $\pm$ 0.001 a	32.40 $\pm$ 0.000 a	101.600 $\pm$ 0.007 a
'Chemlal' in Ain Arnat	59.170 $\pm$ 0.007 a	40.500 $\pm$ 0.001 b	148.700 $\pm$ 0.004 a
'Sigoise' in Ain Azel	78.480 $\pm$ 0.001 a	49.300 $\pm$ 0.001 c	1970.000 $\pm$ 0.100 b
'Sigoise' in Ain Arnat	71.170 $\pm$ 0.020a	45.7 $\pm$ 0.004 bc	1330.500 $\pm$ 0.030 c
Ascorbic acid	n.d.	n.d.	15.500 $\pm$ 0.000 d
Butylated hydroxytoluene	87.640 $\pm$ 0.001 b	n.d.	n.d.



**Table 3.** Significant correlations between biochemical parameters, maturity index, and location in olive samples

Parameters	Pearson correlation ( <i>r</i> )	Significance ( <i>p</i> , two-tailed)	Strength of correlation
Sample / weight	0.807	< 0.001	Strong
Sample / total phenolics	0.700	0.011	Moderate
Location / maturity index	0.839	0.001	Strong
Total pheolics / total antioxidant capacity	0.806	0.002	Strong
Total pheolics / DPPH assay	0.649	0.022	Moderate
Tannins / maturity index	0.719	0.008	Strong
Total antioxidant capacity / weight	0.775	0.003	Strong
Attack rate / weight	0.901	< 0.001	Strong

### Correlation analysis

Correlation analysis evaluated the relationships between biochemical traits (total phenolic content, flavonoid content, and tannin content), antioxidant activity (DPPH and TAC), and environmental factors (location and attack rate) in olive samples (Table 3). Strong positive correlation was found between tannin content and MI and between total phenolic content and MI indicating a close relationship with MI. DPPH and TAC activity also correlated significantly with total phenolic content, highlighting the influence of phenolic compounds on antioxidant properties.

A moderate negative correlation was observed between attack rate and location, suggesting higher attack rates in certain locations. Additionally, a strong negative correlation between tannin and total flavonoid content indicated an antagonistic relationship between these compounds in the olive varieties studied. Correlation analysis evaluated the relationships between various biochemical traits (total phenolic content, flavonoid content, and tannin content), antioxidant activity (DPPH and TAC), and different environmental factors (location and attack rate) in olive samples. The results demonstrated significant correlations among several parameters.

Strong positive correlation was found between tannin content and MI, as well as between total phenolic content and MI. These findings indicate a close relationship between these traits and the environmental variable MI. Furthermore, total phenolic content significantly correlated with DPPH and TAC activity. This suggests that phenolic compounds play a significant role in the antioxidant properties of the olive samples.

### Discussion

Significant variation in fruit characteristics and insect susceptibility between the two olive cultivars and growing locations was found. Fruit weight showed no statistically significant difference between the sites, but cv. ‘Sigoise’ fruits were consistently heavier than cv. ‘Chemlal’. Similar results were obtained in a study with five Oued-Souf region olive cultivars, where the heaviest fruits were from cv. ‘Sigoise’, and the lightest fruits from cv. ‘Chemlal’ (Acila et

al. 2017). This cultivar-specific characteristic indicates that genetic traits of cv. ‘Sigoise’ promote development of larger fruit sizes. These results confirm that different varieties typically exhibit distinct qualitative characteristics.

The attack rates of *B. oleae* differed among various olive samples, suggesting that each cultivar at different locations had a distinct susceptibility to insect attacks. Cv. ‘Chemlal’ in Ain Arnat appeared to be the most resistant, while cv. ‘Sigoise’ in Ain Arnat was the most susceptible. Several previous studies show that certain olive cultivars, such as ‘Coratina’ and ‘Frantoio’, demonstrate significant resistance to *B. oleae* due to their high phenolic content (Iannotta et al. 2016; Caleca et al. 2019). In contrast, more susceptible cultivars tend to have lower levels of protective secondary metabolites in their leaves and fruits. The differences in attack rates could stem from genetic characteristics of each cultivar, as previous studies suggest that olive trees respond differently to fruit fly infestation based on their genotype. The feeding by *B. oleae* activates pathways involved in defense and oxidative stress responses, with resistant varieties potentially possessing more robust mechanisms to limit damage. These genetic traits may explain why ‘Chemlal’ (C2) showed greater resistance than ‘Sigoise’ (S2), highlighting the importance of genotype-specific defense strategies (Karakoyun, Akça Uçkun 2022). The moderate attack rates of *B. oleae* for both cultivars in Ain Azel suggest that both varieties are exposed to similar levels of insect pressure at this site. Studies on the olive fruit fly *B. oleae* have found that environmental variables such as temperature, water content, and landscape complexity can significantly influence insect infestations, but microclimatic factors in higher-altitude regions may reduce pest development (Rondoni et al. 2024). These variations in attack rates highlight the importance of both genetic and environmental factors in shaping plant defense mechanisms against insects. The attack rate by the insect had the strongest and most significant positive correlation with fruit weight, suggesting that the insect preferentially attacks heavier fruits, which is in accordance with literature data (Wang et al. 2009).

Genetic factors strongly influence the biosynthesis of biochemical components in plants (Cheng et al. 2017).

Polyphenol content varied significantly between cultivars, with cv. 'Sigoise' showing higher values, particularly in Ain Azel. The findings of this study contradict those from the West of Algeria, where cv. 'Chemlal' was found to be richer in phenolic content than cv. 'Sigoise' (Dekdouk et al. 2015; Djelloul et al. 2020). The lack of significant difference in flavonoid content between cultivars but existence of significant differences between locations underscores the influence of climate and geographical origin on these active substances. Furthermore, horticultural practices such as fertilization, irrigation, and pruning impact fruit phenolic levels (Gitonga et al. 2022).

However, the condensed tannin content did not follow the same pattern. The highest condensed tannin content was observed in cv. 'Chemlal', specifically from Ain Arnat. This variation suggests that while cv. 'Sigoise' tended to have higher phenolic and flavonoid contents, cv. 'Chemlal', particularly from Ain Arnat, accumulated more condensed tannins, indicating that different genotypes and locations influence specific secondary metabolites differently (Moura de Melo et al. 2023). Correlation results showed a strong positive correlation between condensed tannin content and the maturity levels of the fruit, proving that tannin synthesis is closely linked to the developmental stage of the fruit. A similar trend was also reported from another study (Pavithra, Banu 2017), indicating that as fruits mature, tannin biosynthesis is upregulated, possibly as a response to physiological changes within the fruit. This relationship is likely driven by a combination of biochemical, physiological, genetic, and environmental factors.

A diet abundant in phenolic compounds has been associated with many health benefits. These compounds can diminish oxidative stress, scavenge free radicals, chelate metal ions, and regulate intracellular signaling pathways (Rudrapal et al. 2022). These properties render the olive fruit effective and advantageous for various nutritional and pharmaceutical applications. Analysis of antioxidant capacities in olive fruit extracts using three different assays provided a multi-faceted view of their potential to neutralize free radicals. The extracts were evaluated against established standards, butylated hydroxytoluene and ascorbic acid.

Previous studies suggested that insect attacks may affect different aspects of olive fruits. For instance, it was found that *B. oleae* attacks on cv. 'Rougette de Métidja' olives, when separating healthy from damaged fruits, led to a significant reduction in total phenolic content, including compounds like oleuropein, verbascoside, luteolin-7-O-glucoside, tyrosol, and hydroxytyrosol (Medjkouh et al. 2016). These reductions directly affected antioxidant and antibacterial activity, with healthy olives displaying more potent antibacterial and antioxidant properties than the attacked ones (Medjkouh et al. 2016). In contrast, the present study did not separate healthy and damaged fruits; the olive samples were analyzed as they were, reflecting real

field conditions; thus, no significant relationship was found between the rate of insect attacks and key phytochemical components such as TPC, TFC, and CT. None of these components showed statistically significant correlations with the attack rate. Additionally, the attack rate only displayed weak correlation with antioxidant activity. This lack of a clear relationship may be due to the insufficient intensity of the insect attack.

TPC positively correlated with both DPPH and TAC. Additionally, TC content also positively correlated with DPPH scavenging ability, indicating that the extracts richest in phenolics demonstrated lower antioxidant capacity. The results underscore the superior performance of cv. 'Chemlal', particularly in Ain Azel, despite having the lowest phenolic content. The significantly lower  $IC_{50}$  and  $EC_{50}$  values for this sample in the DPPH and FRP assays, respectively, indicate their strong scavenging ability and reducing power, which surpassed synthetic antioxidant butylated hydroxytoluene, and natural antioxidant ascorbic acid.

The antioxidant compounds found in plant extracts serve various functions, and their activity and mechanism of action heavily depend on their composition and environmental conditions, as these conditions influence the synthesis of plant chemicals with antioxidant properties (Zargoosh et al. 2019). It was found that the antioxidant activity of olive extracts is not solely determined by phenolic content; rather, the specific phenolic profile plays a crucial role (Benavente-García et al. 2000). Previous studies show that some individual phenolic compounds or fractions, particularly if present in a higher ratio in the overall phenolic content, could contribute more to the antioxidant properties of olives than others. It could also be attributed to the synergy between bioactive components (Ljevar et al. 2016; Salem et al. 2020). The consistent superior performance of olive fruit extracts from cv. 'Chemlal' from Ain Azel across all assays suggests that it has the most robust antioxidant profile. It can be concluded that all plant groups have the potential for antioxidant activity. However, the complexity of ecological and genetic factors led to varying chemical processes within the plant. As a result, it is likely that different compounds with distinct antioxidant potentials are synthesized in different regions. Interestingly, this concept could be extended to natural product drug discovery by studying the relationship between the whole metabolome of natural-derived remedies and their biological effect (Ljevar et al. 2016; Salem et al. 2020).

## Conclusions

The assays and measurements revealed notable differences in maturity, weight, attack rates by *Bactrocera oleae*, phenolic content, and antioxidant capacity between the 'Chemlal' and 'Sigoise' olive cultivars, shaped by their growing locations. 'Chemlal' fruits from Ain Azel, in

particular, show higher antioxidant potential, lower EC<sub>50</sub> values, and good resistance to insect attacks, likely due to strong biochemical defense. The Ain Arnat environment promotes a slight increase in fruit weight for both cultivars, with cv. 'Sigoise' consistently weighing more. The findings suggest that cv. 'Chemlal' outperforms 'Sigoise' in antioxidant capacity and pest resistance, emphasizing the role of varietal selection and environmental factors in enhancing olive quality. Optimizing growing conditions could further improve these traits, important for health-related applications. Future research should investigate the environmental and genetic factors affecting phenolic content, antioxidant capacity, and pest resistance, with a focus on metabolomic profiling and post-harvest impacts on these qualities.

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