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Effect of *Bactrocera oleae* infestation on bioactive compounds and antibacterial activities of olive oil

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ABSTRACT

The attack of the olive fly *Bactrocera oleae* can cause severe detrimental effects in bioactive compounds and therefore in olive oil (OO) functional properties.
This study aimed to verify if the attack by *B. oleae* in different rates affect the total phenolics and flavonoids content and consequently the antibacterial properties of OO.
Olive fruits from *Limli* and *Rougette de Métidja* cultivars were hand harvested, in Takerietz (Bejaia), Algeria. A liquid-solid extraction was carried out to obtain the phenolic extract. The total phenolics and flavonoids contents were assessed spectrophotometrically. The antibacterial activity of the OO extracts was evaluated against eight enteropathogenic bacteria.
The lowest total phenolics (894 and 692 mg GAE/kg) were found in OO with the highest rates of attack. There are also significant differences between cultivars. Regarding flavonoids, the losses in *Limli* were lower than in *Rougette de Metidja.* OO extracts revealed antibacterial activity against *E. coli, P. aeruginosa, A. baumannii, S. aureus, B. subtilis,* and *L. innocua.* The healthy samples have a high antibacterial activity comparatively to the attacked ones.
Overall, *B. oleae* attack caused losses in phenolic compounds, implying a decrease in antioxidant capacity. Hence, the antibacterial activity was also affected by the fly attack.

1. INTRODUCTION

OO has a great economic importance in Mediterranean countries and in new producing countries in America, Africa, and Australia [1]. OO consumption is related with several health benefits, namely the prevention of cardio and cerebrovascular diseases, diabetes mellitus, metabolic syndrome, certain cancers, and neurodegenerative diseases. This protective effect is related to OO high levels in bioactive compounds, namely phenolic compounds. Additionally, several studies reported the inhibition or delay of the growth rate of a range of bacteria, related with OO phenolic compounds content [2]. Phenolic compounds as hydroxytyrosol and oleuropein have been shown antimicrobial activity.

Recently, Laincer and colleagues (2014) showed that among the bacteria tested, *Staphylococcus aureus* and, to a lesser extent, *Bacillus subtilis* presented high sensitivity to phenolic extracts obtained from OO of eleven Algerian varieties [3].
The olive fruit fly *B. oleae* is the most serious insect pest of the cultivated olive fruits in the world. It affects the olive tree cultivation, causing severe qualitative and quantitative consequences with economic impact and OO quality losses. The degree of the attack of the drupe by the fruit fly *B. oleae* is strongly related to the OO quality [4].
The aim of this study was to evaluate the effect of the different rates of olive fly attack in two Algerian olive cultivars (*Limli* and *Rougette de Metidja*) on total phenolics and flavonoids content and therefore the antibacterial properties of the OO obtained.

2. MATERIAL AND METHODS

2.1. Samples collection and preparation

Olive fruits from *Limli* (**L**) and *Rougette de Métidja* (**R**) cultivars were hand harvested, in a grove located in the olive production station, Takerietz (Bejaia), Algeria. After harvesting, the attack rate by *B. oleae* was determined [5]. Sampled olive fruits were grouped into healthy olives (not attacked - **S**); non selected olives (reflecting the real attack rate on the fruits **- N**); only attacked olives (every olive had at least one exit-hole **- A**).
OO was extracted using a laboratory mill equipped with a blender and a centrifuge.

2.2. Phytochemicals quantification

Phenolic compounds were obtained by a liquid-solid extraction using a SPE C_{18} column [6]. For total phenolics evaluation, a spectrophotometric method was applied. Briefly, Folin-Ciocalteu reagent (1:10) was added to the extract (0.2 mL). After 2 min at room temperature, a solution of sodium carbonate (75 g/L, 0.8 mL) was added. After 30 min, the absorbance was read at 765 nm. Results were expressed as mg Gallic Acid Equivalents (GAE)/kg of oi l[7].
For total flavonoids content determination, 1 mL of diluted extracts was added to a methanolic solution of $AlCl_3$, 2% (1mL). After 10 min, the absorbance was read at 430 nm. Results were expressed as mg Quercetin Equivalents (QE)/kg of oil [8].

2.3. Antibacterial activity

The antibacterial activity of the sample extracts was evaluated against eight referenced human enteropathogenic bacteria: *Escherichia coli, Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumonia* (Gram negative strains); *Bacillus subtilis, Staphylococcus aureus, Listeria innocua,* and SARM (*Staphylococcus aureus* Resistant to Methicillin) (Gram positive strains). The antibacterial activity was evaluated using the agar disc diffusion method, with some modifications [9]. The bacterial strains

were firstly grown in Muller Hinton medium (37 ºC; 18-24 h). The inocula were transferred to a physiological suspension medium and adjusted to 0.5 McFarland turbidity standard. Sterile discs, impregnated with 10 µL of each extract were placed in the infusion agar containing bacteria. Firstly, Petri dishes were kept at 4 ºC during 1 h, and finally incubated at 37 ºC during 24 h. The antibacterial activity was assessed by measuring the difference between the diameters of the zone of inhibition and the diameter of the disc, expressed in mm.

3. RESULTS AND DISCUSSION

Total phenolics and flavonoids contents of OO with different fly attack rates of the two studied cultivars are presented in Figure 1. The lowest phenolics content was found in OO with the highest rates of fly attack. There are significant differences between cultivars, with total phenolics ranging from 1140 to 894 mg EAG/kg in *Limli* cultivar and from 1009 to 692 mg EAG/kg of oil in *Rougette de Metidja* cultivar, for healthy and unhealthy samples, respectively (Figure 1). *Rougette de Metidja* cultivar was more affected by the fly attack, therefore, is the cultivar more sensitive. The decrease of total phenolics content in attacked samples may be related to the increase of the polyphenol oxidase activity due to the presence of oxygen that reaches the pulp by the exit-holes made by fly larvae [10].

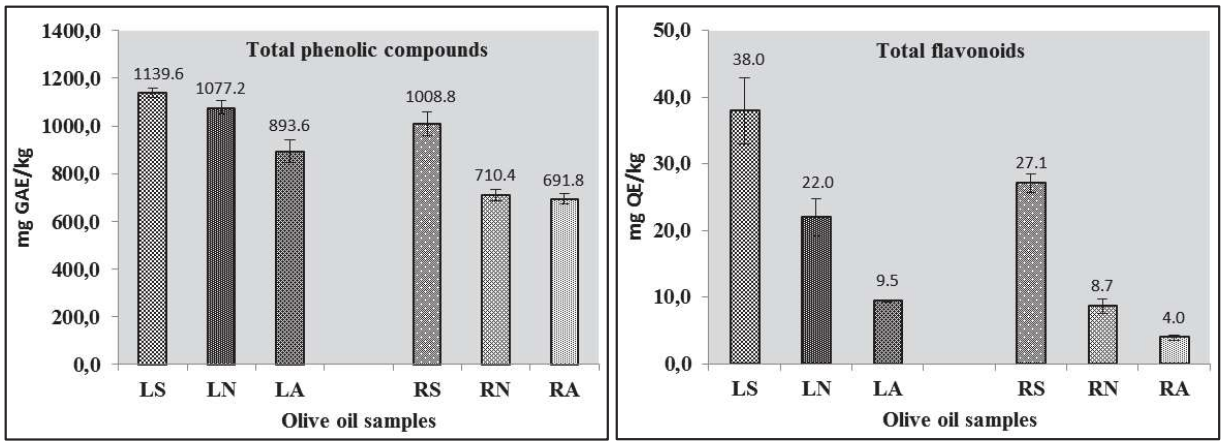

Figure 1. Total phenolic compounds (mg GAE/kg) and flavonoids (mg QE/kg) of olive oil obtained from *Limli* (L) and *Rougette de Metidja* (R); S - sain (healthy), N - natural, A - 100% attacked.

The attack of olives by the *B. oleae* also caused flavonoids loss (Figure 1). The losses verified in *Limli* OO were also lower than the verified in *Rougette de Metidja*.

The present study aimed to verify the OO phenolic compounds action against bacteria. Therefore, the antibacterial activity of OO extracts from healthy and attacked samples against eight bacteria was tested (Table 1). The results showed that OO extracts (2.8 mg/mL), revealed antibacterial activity against *E. coli, P. aeruginosa, A.baumannii* (Gram negative microorganisms) and *S. aureus, B. subtilis* and *L. innocua* (Gram positive microorganisms). The referred extracts were inactive against SARM and *K. pneumoniae.* The healthy samples have a high antibacterial activity comparatively to the attacked ones.

Table 1. Antibacterial activity of OO extracts obtained from *Limli* (L) and *Rougette de Metidja* (R).

	LS	LN	LA	RS	RN	RA
E.coli	20.1±0.10d	13.8±0.20b	11.0±1.00a	15.5±0.05c	15.5±0.50b	10.9±0.50a
P. aeruginosa	14.10±0.60c	9.25±0.25b	7.35±0.35a	10.05±0.45b	7.85±0.75a	nd
A.baumannii	14.00±1.00c	10.95±0.25b	9.60±0.40a	18.35±0.35d	11.65±0.35b	10.50±0.50ab
K. pneumoniae	nd	nd	nd	nd	nd	nd
S. aureus	16.50±0.50c	12.50±0.50b	nd	16.00±0.00c	11.75±0.75b	9.50±0.50a
B. subtilis	16.15±0.15e	11.95±0.55c	8.50±0.50a	14.80±0.50d	10.95±0.15b	8.15±0.15a
L.innocua	15.75±0.75e	12.15±0.55c	9.90±0.70a	13.95±0.35d	11.30±0.70bc	10.30±0.30ab
SARM	nd	nd	nd	nd	nd	nd

S - sain (healthy), N - natural, A -100% attacked. nd- not detected.
Means followed by different letters are significantly different (P<0.05).

4. CONCLUSIONS

Olive fruit fly attack affects the OO total phenolics and flavonoids contents and consequently the antibacterial activity. Considering the two varieties studied, *Rougette de Metidja* is the more susceptible. However, in both, pejorative effects were observed. Earlier harvesting could be a preventive approach to reduce the *B. Oleae* damage and improve the OO quality.

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