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PHENOLICS COMPOUNDS OF OLIVE AND OLIVE LEAVES IDENTIFIED IN THE RESISTENCE TO *PRAYS OLEAE* (BERNARD)

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Abstract

To demonstrate the resistance of olive trees against *Prays oleae* (Bernard), we study the extraction and analysis by HPLC of synthesis phenolic compounds in the olive and olive leaves of *Oleae europeae* infested by Prays oleae. The HPLC analysis distinguished some differents phenolic compounds between olive and olive leaves according to their chromatographic and spectral characteristics into two new componds like rutin and tannin with others phenolics componds include in the defense to *Prays oleae* namely, cafeic, verbascoside, oleuropein and luteolin.

Key words : *Oleae europeae*, *Prays oleae*, phenolics compounds, resistance, insects.

Introduction

The olive tree, *Olea europea* is in full expansion in many countries. Despite its importance, it faces several diseases that severely affect its tree production (Santos *et al*., 2013), where the olive moth, *Prays oleae* (Bernard) (Lepidoptera : Praydidae) is considered one of the most important olive pests (Villa Serran, 2016). *Prays oleae* develops three generations per year. Larval stages feed on different organs of the olive tree. Eggs of the anthophagous generation are laid on floral buds and, after hatching, larvae feed on the flowers. The flight period of adults occurs at the end of spring, laying the eggs on the olive calyx and larvae of the carpophagous generation, bore into the olive stone and feed on the seed. At the end of summer and beginning of autumn, adults emerge and lay the eggs of the phyllophagous generation on the olive leaves. Larvae of the phyllophagous generation dig galleries and feed on leaves, where they overwinter till the beginning of spring (Arambourg, 1986).

The phenolic compounds constitute the molecules often implied in plant defence to pathogens and associated with the plant host resistance (El Modafar et El Boustani, 2005). Phenolic secondary metabolites, which are involved in the special organoleptic properties of oil, have been shown to play a role in the resistance of some olive (*Olea europea* L.) varieties to oil autoxidation (Botia *et al*., 2001). Thus, increasing the endogenous levels of these secondary metabolites can improve the resistance properties of the plant and can be used as a natural alternative for preventing plant diseases. Methods for detecting and recognizing phenolic compounds rely mainly on chromatographic separation, using HPLC analyses (El Modafar et El Boustani, 2001), which allow their successful identification.

In Algeria, little is known about the resistance of *Oleae europea* L to insects. The aim of this work is to detect the phenolic compounds potentially present in the defense of olive trees with the chemical nature of these compounds using HPLC method.

Materials and Methods

Yields extraction

The olives and olive leaves were washed and dried with paper towel; they were cut into approximately 1 cm squares, dried in an oven at 60°C for at least 24 h, crushed and degreased in a soxhlet, before use. All analyses were conducted in triplicate, and the results were based on dry weight per 100 g for each sample.

Tannins extraction

Powdered materials (100 g) was extracted at 4°C using 500 ml of a mixture of acetone-water (25/45, v/v)

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for 4 days,separately (Bruneton, 1999).

The extracts were filtered under vacuum through filter paper and the acetone was evaporated under reduced pressure. Subsequently, dichloromethane (2 × 25 ml) was used for the extraction of lipids and pigments from the aqueous extracts using a separating funnel. Afterward, the aqueous phase was extracted with 25 ml of ethylacetate. This process was repeated four times. After filtration, the organic phases (ethylacetate) containing tannins were recovered and concentrated to dryness under vacuum, using a rotary evaporator. The residue obtained after evaporation was kept at 4°C and used for further investigation.

Flavonoids extraction

A quantity of 10 g of dried material was extracted with 100 ml of methanol and 5 g of calcium carbonate by boiling for 1 h,separately (Danguet and Foucher, 1982). After filtration, throughWhatman filter paper, the methanol was evaporated under reduced pressure to eventually give an aqueous extract. Subsequently, the dry extract was recovered with 50 ml of boiling water. The aqueous extract was filtered and subjected to solvent fractionation; first l with diethylether, then ethylacetate and finally n-butanol, using separating funnel (pyrex). All fractions were concentrated, dried to constant weight in an oven at 45°C and kept at 4°C.

Extraction of alkaloids

An amount of 10 g of dried samples was mixed with 250 ml of HCl 2% and 110 ml of ethylacetate, separately. After cold soaking (4°C) for 10 h, the mixture was filtered and basified with NH_4OH. The basic aqueous phase was extracted twice with ethylacetate until no alkaloid was detected in the aqueous phase. The alkaloid residue was obtained by decantation and evaporation of the organic phase (Bruneton, 1999).

Plant extraction

The dried powder of olives and olive leaves (10 g) was extracted in triplicate separately, using EtOH (96% v/v) at room temperature, under stirring. The aqueous suspension of the concentrated EtOH extract was evaporated to dryness and used for all investigations (Kukic *et al*., 2008).

Determination of total phenolic content

The amount of total phenolic content was determined by Folin-Ciocalteu procedure (Singleton and Rossi, 1965). Aliquot (0.1 ml) of each sample extract was transferred into the test tubes and their volumes were made up to 3 ml with distilled water. After addition of 0.5 ml Folin-Ciocalteu reagent and 2 ml of 20% aqueous sodium carbonate, tubes were vortexed and incubated at room temperature under dark condition. The absorbance was recorded after 1h at 650 nm JEN WAY 6405 UV/Vis spectrophotometer. The total phenolic content was calculated as a Pyrocatechol equivalent (mg PE/g DW).

High performance liquidchromatography (HPLC)

Total phenolics analyses on methanolic extract of infected olives and infected olive leaves were carried out using Jasco HPLC, separately. It consists (Jasco HPLC) of a pump (PU-2089 Plus) and UV detector model UV-2077 with ChromNAV on a XBridgean alytical column (RP-C18 : 5 µm, 4.6 × 150 mm) (Waters Inc. USA), having gradient solvent system and parameter condition. The chromatograms were observed at wavelengths of 254, 270, 280 and 329 nm. All the analyses were carried out at sample concentration of 1 mg/ml and injection volume of 20 µl.

Results and Discussion

Total phenol content

Fig. 1 present the total phenol from uninfected and infected olive. Total phenol contents in the infected olive (93 mg/g) were higher than those measured in the uninfected olive(58mg/g). The polyphenols content in uninfected and infected leaves were presented in fig. 2. Total phenol content in infected olive leaves is (57 mg/g) compared to phenol centent in uninfected olive leaves (24.12 mg/g).

Yields extraction

The yields of tannins, flavonoids and alkaloids of olive was presented in fig. 3. The yield of tannins in olive from infected and uninfected samples was 2.2 and 3.5%, respectively. The yield of flavonoids and alkaloids was higher in infected olives: 6.4 and 8.7% for flavonoids and 2.6 and 3.8% for alkaloids content in uninfected and infected samples, respectively.

Fig. 4 present the yields of tannins, flavonoids and alkaloids of olive leaves. The yield of flavonoids in whole leaf from infected olive plants was 4.01% followed by those of alkaloids and tannins 3.22% and 1.85, respectively.

Identification of phenolic compounds by HPLC

The data (retention time, λ max in the visible region, and tentative identification) obtained for the phenolic compound peak in the HPLC-analyses of olive and olive leaves are presented in figs. 5 and 6, respectively. HPLC studies point to four phenolic compounds determined in olive extracts: Cafeic acid (*tR* = 1.93 min, maximum absorbance at 245 nm), Tannic (*tR* = 18.41 min, maximum

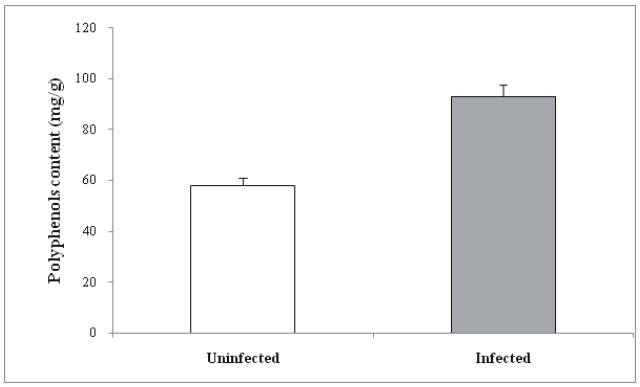

Fig. 1 : Polyphenols content of uninfected and infected of olive.

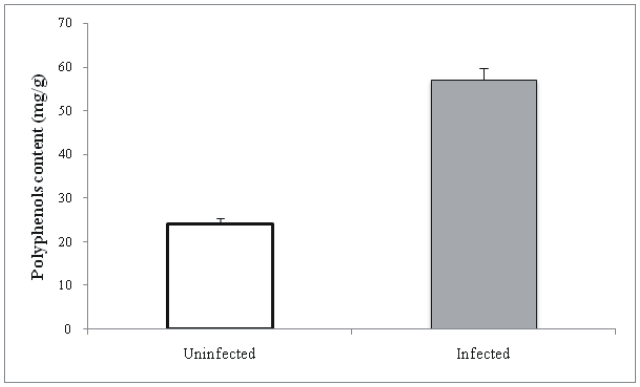

Fig. 2 : Polyphenols content of uninfected and infected of olive leaves.

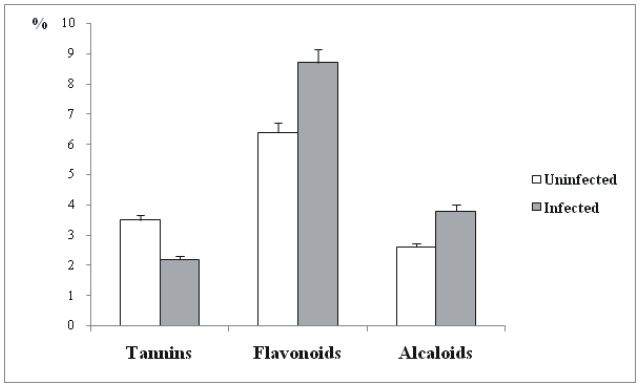

Fig. 3 : Tannins, flavonoids and alkaloids contents of uninfected and infected olive.

absorbance at 250 nm), Rutin (*tR* = 19.33 min, maximum absorbance at 249 nm) and Verbascoside (*tR* = 19.74 min, maximum absorbance at 248 nm).

In olive leaves extracts, five phenolic compounds determined with HPLC studies : caffeic (*tR* = 1.63 min, maximum absorbance at 243 nm), Verbascoside (*tR* = 1.96 min, maximum absorbance at 240 nm), tannic (*tR*= 17.03 min, maximum absorbance at 241 nm), oleuropein (*tR* = 18.53 min, maximum absorbance at 231 nm) and luteolin (*tR*=19.94 min, maximum absorbance at 240 nm).

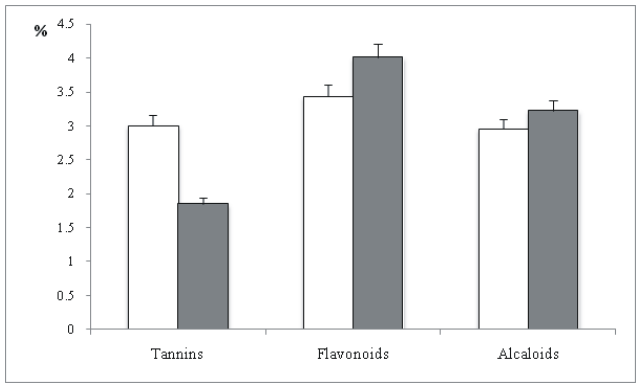

Fig. 4 : Tannins, flavonoids and alkaloids contents of uninfected and infected olive leaves.

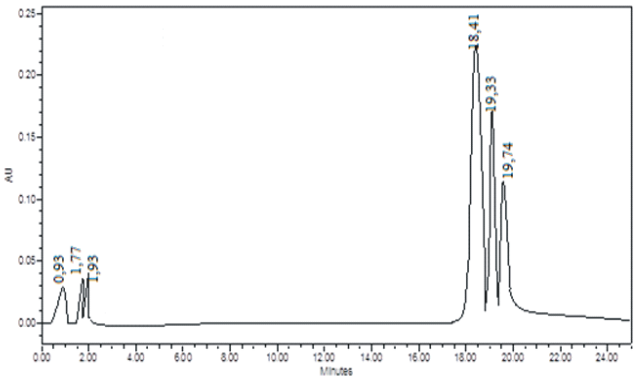

Fig. 5 : Chromatogram (zoom) recorded at 254 nm showing the phenolic compounds profiles identified and not identified of olive (*Olea europea* var. Sigoise).

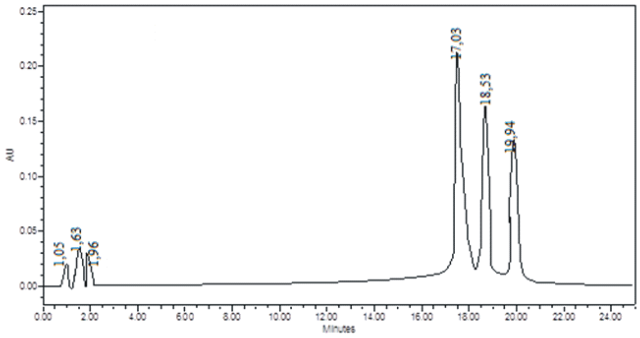

Fig. 6 : Chromatogram (zoom) recorded at 254 nm showing the phenolic compounds profiles identified and not identified of olive leaves (*Olea europea* var. Sigoise).

Discussion

The obtained results show that total polyphenols were present in infected olive and olive leaves at higher levels than in uninfected samples. El Boustani *et al*. (1998) demonstrated that the inoculation of the olive twigs by a conidial suspension of *Verticillium dahlia* resulted in important modifications in flavone and phenol levels. These findings suggest that the first step of the response mechanism to infection in olive plants is a rapid accumulation of phenols at the infection site, thus reducing

or slowing the pathogen growth, as reported for other vegetal materials (Del Rio *et al*., 2004).

The olive leaves infected by sooty mold demonstrate the presence of several phenolics compounds (Ilias *et al*., 2015). Therefore, in contrast to flavonoids and alkaloids, the tannin content of the uninfected samples was higher than that of the infected one. Corbaz (1990), whose study results show that the young leaves at the cotton plant are often resistant to *Verticillium dahlia* and become sensitive as they grow older.

In selective extractions, those concentrations of alkaloids in infected olive plants were higher than in uninfected ones also suggest that alkaloids may have a role in the response mechanism of olive plants to sooty mold. These results are similar to that found by Ilias *et al*. (2015), who found olive infected by sooty mold. Baidez *et al*. (2007) suggest a strong role of the phenolic compounds in the olive tree defense particularly, oleuropein and tyrosol to *Verticillium dahliae*.

Zine EL Aabidine *et al*. (2010), demonstrate that the resistance of olive tree to *Spilocaea oleagina* was related to multifactorial phenolic components,the tyrosol and its derivatives were in relation to constitutive resistance, whereas the oleuropein and rutin were in relation to induced resistance.

Our main findings were that the HPLC analyses revealed the presence of some phenolic compounds in infected olive, namely rutin. Ilias *et al*. (2015) found tannin for the first time in olive leaves infected by *Verticillium dahliae*. In this study, we have found that rutin have a role in the defense mechanism to *Prays oleae*.

For olive leaves, several works reported the abundance of these phenolic compounds in the olive tree leaves, particularly the luteolin-7-glucoside, the verbascoside and the oleuropein (Ryan and Robards, 1998; Silva *et al*., 2006; El-Hassani *et al*., 2009; Rahioui *et al*., 2009). HPLC analyses demonstrated the presence of five compounds, oleuropein acid, verbascoside, caffeic acid, tannin and luteolin. We suggest that these compound have a role or function in the resistance to insects.

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