Consequence of Water Deficit on Biological Activities of Olive Extract (Olea europaea L.) Growing in Tunisia

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Received: 10 October 2024 / Received in revised form: 25 February 2024, Accepted: 07 March 2024, Published online: 15 March 2024
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Abstract

The consequence of water stress on the polyphenolic content, anticholinesterasic and antifungal activities of methanolic leaf extracts of three Tunisian (Olea europaea L.) varieties: Meski, Oueslati, and Jarboui was investigated. Our results showed that total polyphenolic and flavonoids compounds amplified in all tested varieties under water stress. In addition, water stress increased the anticholinesterasic activity in all all olive cultivars. But Oueslati variety had the best one with the lowest IC₅₀. Antifungal activity of all extracts of olive leaf generally increased under water deficit. We can conclude that water stress exposed positive effect on secondary metabolites; hence the biological effect of olive leaf of different varieties. We can conclude that water stress induced an increase in biological activities (antifungal and anticholinesterasic effects). In addition, Ouslati cultivar may be considered as the most tolerant one, followed by Jarboui and Meski was the most sensitive. Ouslati cultivar may be considered as the most tolerant one, followed by Jarboui and Meski was the most sensitive.

Keywords: Olive, Antifungal activity, Water deficit, Phenols, Anticholinesterasic activity

Introduction

Olea europaea L. are part of the Oleaceae family and is native to tropical and subtropical regions (Sorkheh & Khaleghi, 2019). It is resistant to water deficit and salinity (Acar-Tek & Ağaçgündüz, 2020) and its great capacity for adaptation to drought is manifested by morphological and physiological leaf modifications (Boss et al., 2019). The leaf contain high amount of secoiridoid derivatives like oleuropein and oleacein, known by their hypoglycemic and hypotensive effects (Okorie et al., 2019), in Tunisia Olive trees traditionally grow in drought conditions (Edziri et al., 2020). However, responses to stress conditions differ between varieties.

The antioxidant, antimicrobial, cytotoxic, hypoglycemic, antiviral effects of olive leaves have also been investigated (ALHaithloul et al., 2019; Techathuvanan et al., 2019).

According to our knowledge there is no research documenting the impact of water stress on phytochemical composition, anticholinesterasic and antifungal activities of olive leaf extracts. Therefore the goal of this scientific research was to investigate the consequence of water deficit on some morphological parameters, phenolic compounds, anticholinesterasic and antifungal effect of methanolic leaf extracts of three Tunisian olive varieties (Oueslati, Jarboui and Meski).

Materials and Methods

Site Description

The effect of water stress on three Tunisian olive varieties (Olea europaea L.) cv. Meski Jarboui and Oueslati were evaluated. three years old olive plantlets, were full-grown in greenhouse into 20 dm³ pots. During the experience the temperature and humidity were 25/32°C and 65/55%, respectively. for the control plants we used six plants and which are irrigated once a week to the capacity of the field. Further six plants from respectively cultivar devoid of water during May and June by withholding water.

Leaf Morphology and Sclerophylly

The analyses were performed on twenty adult leaves torn from each variety of each cultivar as described by Edziri et al. (2020).

Methanol Extract Preparation

150 mg of desiccated leaves were macerated with 20 mL of MeOH (80 %) for 30 min and shaking at 200 rpm. The extracts were evaporated with rotary vapor after filtration (Edziri et al., 2019) and finally kept at -4°C for biological test.

Determination of Polyphenolic Compounds

The content of total phenolic compounds (TCPT) was determined with the Folin-Ciocalteu reagent (Wang et al., 2022). 100 μL of crude extract or standard was mixed with 500 μL of 10% (v/v) Folin-Ciocalteu reagent. The mixture was placed in the dark for 3 min before adding 400 μL of 7.5% (w/v) Na₂CO₃. The mixture was incubated in the dark for 30min and the absorbance was measured...
at 731 nm using a spectrometer. TCPT was expressed as gallic acid equivalent (EqAG)

**Determination of Flavonoids Content**

To determine flavonoid content, colorimetric method was used as established by Edziri et al. (2020) and it is calculated in terms quercetin equivalent (EqQ) per gram extract.

**Evaluation of Acetyl Cholinesterase (AChE) Inhibition Effect**

This activity was determined as defined by Ataie et al. (2019), 50 μL of buffer, was mixed with 20 μL of different concentrations of methanolic extracts after that 25 μL of 0.22 U/mL of AChE enzyme was putted. Next 15 min of incubation at 37 °C, we added 30 μL of acetylthiocholine iodide (15 mM AChI) and 130 μL of S,S'-dithiobis [2-nitrobenzoic acid] (3 mM DTNB) after incubation for 30 min the absorbance was read at 405 nm. IC50 was determined as the concentration of the extract required to inhibit 50% of enzyme.

**Evaluation of the Antifungal Activity**

Four Candida species; American-Type Cell Culture (ATCC) was used. They are numbered as Candida albicans 90028 (C.a), Candida parapsilosis ATCC 22019 (C.p), Candida glabrata ATCC 90030 (C.g), and Candida keuseii ATCC 6258 (C.k). Sabouraud agar was used as a cultural medium.

**Antifungal Potential**

The technique described by Edziri et al. (2020) was used to calculate the essential oils' minimum inhibitory concentrations (MIC). Ten test tubes are required for the dilution procedure, which entails creating a series of tubes with Tryptic Soy Broth (TSB). Nine more tubes hold one milliliter of TSB each, while the first tube holds two milliliters. Subsequently, 20 microliters of an essential oil solution are introduced into the initial tube, and it is diluted to achieve a concentration range of 1 to 0.0039%. Next, 10 μl of the bacterial suspension (final concentration: 107 CFU/ml) is added to each tube for inoculation. The tubes are incubated for 18 hours at 37°C with a control that does not include extract (Moss et al., 2022). The minimum inhibitory concentration (MIC) is the lowest concentration of the essential oil that prevents any growth that is apparent to the unaided eye after 16 to 20 hours of incubation at 37°C.

**Statistical Analyses**

One-way analysis of variance (ANOVA) was carried out followed by Dunnett’s t-test which is used to test significance (P < 0.05).

**Results and Discussion**

**Leaf Morphology and Sclerophyll**

According to Table 1, we can observe that underwater stress decreased LA, SLA, and density, augmented in all tested varieties. We can observe that physiological variations depend on varieties.

**Table 1.** Morphological parameters of Tunisian olive varieties

<table>
<thead>
<tr>
<th>cultivars</th>
<th>LA (mm²)</th>
<th>S (mgH₂O cm⁻²)</th>
<th>SLA (m² kg⁻¹)</th>
<th>D (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watered</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oueslati</td>
<td>387.1a</td>
<td>22.13a</td>
<td>4.42a</td>
<td>306.3a</td>
</tr>
<tr>
<td>Jarboui</td>
<td>426.3a</td>
<td>24.55a</td>
<td>3.14a</td>
<td>460.2a</td>
</tr>
<tr>
<td>Meski</td>
<td>330.4a</td>
<td>20.52a</td>
<td>3.32a</td>
<td>503.5a</td>
</tr>
<tr>
<td>Stressed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oueslati</td>
<td>321.2b</td>
<td>29.57b</td>
<td>4.26b</td>
<td>280.3b</td>
</tr>
<tr>
<td>Jarboui</td>
<td>312.9b</td>
<td>27.54b</td>
<td>2.79b</td>
<td>410.4b</td>
</tr>
<tr>
<td>Meski</td>
<td>230.6b</td>
<td>25.54b</td>
<td>3.15b</td>
<td>470.6b</td>
</tr>
</tbody>
</table>

Values signify means ± standard deviations for six repetitions.

**Polyphenols and Flavonoids Contents**

As shown in Figures 1 and 2 Oueslati had the highest phenols contents although Meski had the weakest concentration of polyphenols and flavonoids. Total polyphenols and flavonoid content augmented in all tested varieties. Under water deficit. A greater quantity of phenols was found in the Oueslati variety tracked by Jarboui and Meski.

**Anticholinesterase Activity**

![Figure 1. Polyphenols content of methanolic leaf extracts of Olea europea varieties.](image)

Values signify means ± standard deviations for six repetitions.

![Figure 2. Total flavonoid content of MeOH leaf extracts.](image)

Values signify means ± standard deviations for six repetitions.
The acetylcholinesterase inhibitory activity of methanolic extracts of olive leaf varieties was determined by the microplate assay. IC₅₀ values are given in Table 2. As exposed, we can conclude that the Oueslati variety had the greatest anticholinesterase activity.

Table 2. IC₅₀ values for acetylcholinesterase (AChE) inhibition of methanolic extracts of olive varieties

<table>
<thead>
<tr>
<th>Methanolic extract of olive varieties</th>
<th>IC₅₀ (mg.ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stressed</td>
</tr>
<tr>
<td>Oueslati</td>
<td>0.10±0.08*</td>
</tr>
<tr>
<td>Jarboui</td>
<td>0.15±0.08*</td>
</tr>
<tr>
<td>Meski</td>
<td>0.21±0.05*</td>
</tr>
<tr>
<td>Physostigmine</td>
<td>0.04±0.01</td>
</tr>
</tbody>
</table>

Results are reported as the means ± SE of 6 independent experiments performed in duplicates. *p < 0.05.

Antifungal Activity

This research work was done to estimate the antifungal activity of methanolic extracts of olive varieties before and under water stress. As shown in Figures 3-5, we can observe that all methanolic extracts of the olive variety had good antifungal activity against all candida species before water stress, with MIC varied between 32 and 64 mg/ml. Under water deficit, the antifungal activity increased with all stressed extract varieties. Methanolic extract of the Oueslati variety showed the best activity with MIC values varying between 16 to 4 mg/ml against candida species.

![Figure 3](image3.png) **Figure 3.** Antifungal activity of methanolic leaf extract of Oueslati a) variety

![Figure 4](image4.png) **Figure 4.** Antifungal activity of methanolic leaf extract of Meski b) variety

Trichomes in *Olea europaea* located on the abaxial side of the leaf, have the role of protecting stomata from UV rays and preventing dehydration (Moss *et al.*, 2022). According to these results, Oueslati leaf has a higher density of stomata and trichomes than the other variety.

We see that the lack of water has decreased in the three varieties, the Oueslati variety seems best adapted to drought thanks to its smaller leaves. On the other hand, smaller sheets allow better control of heat dissipation and minimize water loss through stomata closing. The decrease in the surface area of the leaf is a defense mechanism to avoid dehydration and allows the plant to better resist the lack of water.

The decrease in SLA observed in the leaves of plants subjected to water stress is explained by the difference in density of the leaf (Wang *et al.*, 2022). ‘Oueslati’ had the lowest density. The Meski cultivar showed the lowest values, which shows that this cultivar is more sensitive to drought conditions than other varieties.

The production of Reactive Oxygen Species (ROS) in the chloroplast is one of the first biochemical responses of cells in response to water stress. In this context, photosynthesis is reduced, the plant can no longer use the excess energy provided by light and oxidative stress will occur. There is a production of ROS, by-products of oxygen metabolism (oxygen O₂- ions. OH free radicals, H₂O₂ peroxides), which will trigger a process aimed at eliminating active oxygen species (Boss *et al.*, 2019; Deng *et al.*, 2019).

Inhibition of AChE is a treatment strategy against several neurological disorders like Alzheimer’s disease (Edziri *et al.*, 2012). The methanolic extract of Oueslati variety olive leaves was found to be the most active against acetylcholinesterase during water stress with an IC₅₀ of 0.1 mg/ml. These results confirm well with those described in other research work (Kachoie *et al.*, 2012; Edziri *et al.*, 2019).

We can see that the observed differences in the antifungal effects of olive leaf varieties can be associated with their geographic location, and environmental conditions (Abidi *et al.*, 2019). The response of the plant to environmental conditions and more
particularly to water deficit, which is the most limiting stress on growth, has an impact on leaf structure. It differs depending on the nature and duration of stress (Ganjalikhan et al., 2019). But this research work is in accordance with the work of Kachoie et al. (2012) that confirmed the importance of antimicrobial potential against gram-positive and negative bacteria of the Thymus diagenesis extracts which increased (1000 µg/ml) under water deficit. Leaves may be thicker due to an increase in the thickness of the mesophyll and cuticles, with an increase in dry matter content per surface area and unit of volume (Wang et al., 2022).

In this work, the comparison of the antifungal activity of these three olive cultivars proved that the antifungal activity increased in all olive cultivars in case of water deficit. this increase may correlate with the high polyphenol content as has been described in other research works (Edziri et al., 2019; Wang et al., 2022). But this research work is in accordance with the work of Kachoie et al. (2012) that confirmed the importance of antimicrobial potential against gram-positive and negative bacteria of the Thymus diagenesis extracts which increased (1000 µg/ml) under water deficit.

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Conclusion

In conclusion, our study can be considered as the first report on the impact of water stress on antifungal and anticholinesterasic activities of Oueslati, Meski and Jarboui olive varieties. Our results demonstrated that water stress affects the physiological and biological criterion of Tunisian olive varieties. Oueslati variety appears the most adapted variety to drought and possess the best antibacterial and anticholinesterasic effects, followed by Jarboui and Meski. This research will be continued in a future work of other Tunisian varieties, to have an idea of their mechanism of tolerance to water stress and their biological activities in the face of other stress factors.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

References


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