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

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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 europea L.) varieties: Meski, Ouslati, and Jarbouii 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 IC_{50}. 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 & Ağagündüz, 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 varities (*Oleaeuropaea* L.) cv. Meski Jarboui and Oueslati were evaluated. three years old olive plantels, were full-grown in greenhouse into 20 dm^3pots. 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

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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 (EgQ) per grame 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 5,5'-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

Fungi

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 kreuseii* 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 Sclerophylly

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

cultivars	LA (mm2)	S (mgH20 cm-^{2})	SLA (m^2 kg-1)	D (g kg-1)
Watered				
Oueslati	387.1a	22.13a	4.42a	306.3a
Jarboui	426.3a	24.55a	3.14a	460.2a
Meski	330.4a	20.52a	3.32a	503.5a
Stressed				
Oueslati	321.2b	29.57b	4.26b	280.3b
Jarboui	312.9b	27.54b	2.79b	410.4b
Meski	230 .6b	25.54b	3.15b	470.6b

Values signify the mean of 8 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.

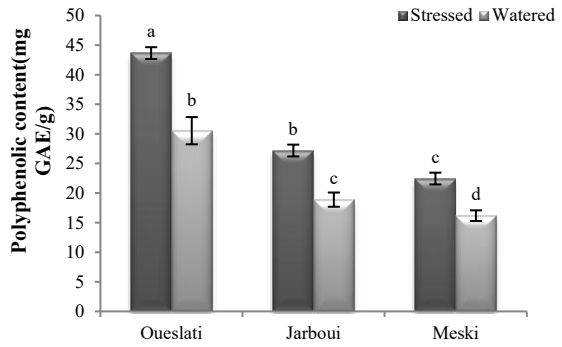

Figure 1. Polyphenols content of methanolic leaf extracts of Olea europea varieties.
Values signify means ± standard deviations for six repetitions.

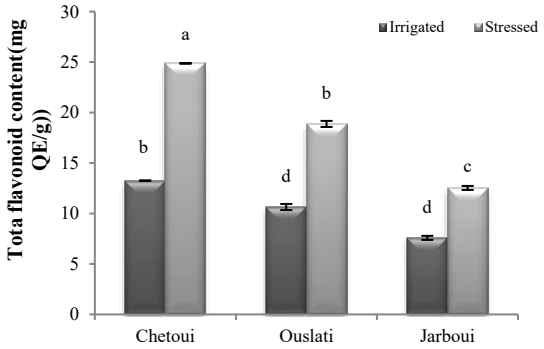

Figure 2. Total flavonoid content of MeOH leaf extracts.
Values signify means ± standard deviations for six repetitions.

Anticholinesterase Activity

The acetylcholinesterase inhibitory activity of methanolic extracts of olive leaf varieties was determined by the microplate assay. IC_{50}

values are given in **Table 2**. As exposed, we can conclude that the oueslati variety had the greatest anticholinesterase activity.

Table 2. IC_{50} values for acetylcholinesterase (AChE) inhibition of methanolic extracts of olive varieties

Methanolic extract of olive varieties	IC_{50} (mg.ml^{-1})	
	Stressed	**Watered**
Oueslati	0.10±0.08*	4.35±0.14*
Jarboui	0.15±0.08*	5.76±0.25*
Meski	0.21±0.05*	3. 09±0.12*
Physostigmine	0.04±0.01	

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.

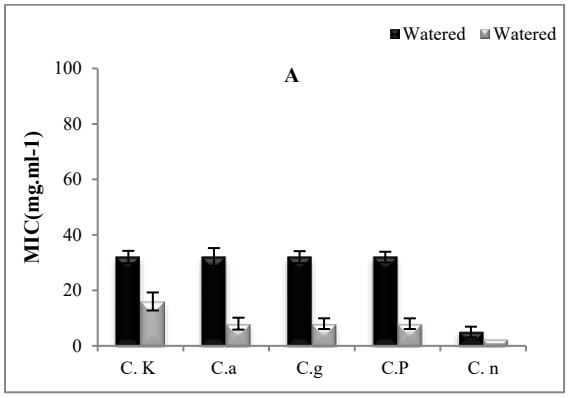

Figure 3. Antifungal activity of methanolic leaf extract of Oueslati a) variety

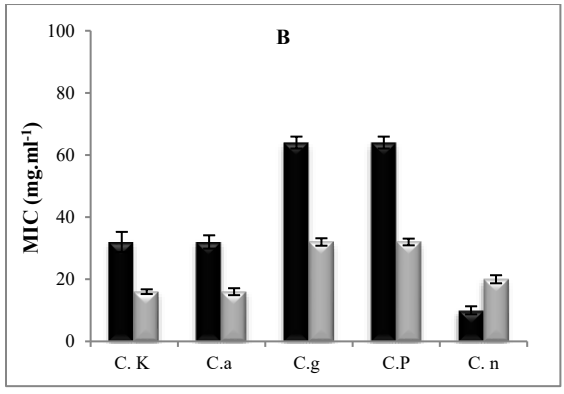

Figure 4. Antifungal activity of methanolic leaf extract of Meski b) variety

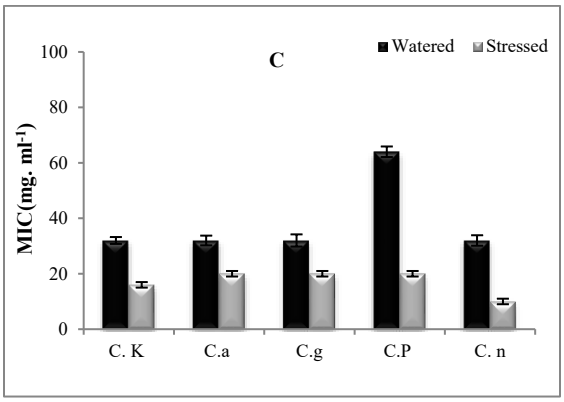

Figure 5. Antifungal activity of methanolic leaf extract of Jarboui c) variety against fungi; MIC: minimal inhibitory concentration.

Trichomes in *Olea europea* 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 in other Tunisian varieties, to have an idea of other varieties and their mechanism of tolerance to water stress and their biological activities in the face of other stress factors.

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