Assessment of protective mechanisms against ultraviolet stress on two species of palms seedling grown under laboratory conditions

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Abstract

The present study was carried out for examining the alterations of total chlorophyll and antioxidants contents (carotenoids, phenols and proline), on two species of palm seedlings, Date palm (*Phoenix dactylifera* L.) and Washingtonian palm (*Washingtonia filifera* induced by exposure to UV-B radiations at different periods (2,4,8 and 10 hrs/day). Results showed that the 30 days of UV-B treatment for 2 and 4 hrs./ day caused significantly increased in total chlorophyll contents of both palm seedling species. While the lowest contents of chlorophyll were obtained in plants grown under UV-B radiation stress for 10 hrs./day. On the contrary, the highest value of carotenoids content was recorded after 8 hrs./day of treatment with UV, then it reduced after treated with 10 hrs./day. Similarly, the content of total phenol and proline showed significantly increased with increasing time of UV-B exposed in both species. The study also revealed a significant difference between two palm species in terms of tolerance to UV-B, where it was found that the Date palm (*Phoenix dactylifera* L.) seedlings were more resistance to such radiation than (*Washingtonia filifera*).

Keywords: Date palm (*Phoenix dactylifera* L.), *Washingtonia filifera*, UV-B, Stress, Chlorophylls, Carotenoids, Phenols, Proline

تقييم آليات الدفاع ضد الارتفاع في الاشعة فوق البنفسجية نوع–ب في نوعين من النخيل النامية تحت الظروف المختبرية

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المستخلص:

أجريت هذه الدراسة لفحص التغيرات في المحتوى الكلي للكلوروفيل ومضادات الأكسدة (الكاروتينويد ، الفينولات والبرولين) في نوعين من شتلات النخيل هي نخيل التمر (*Phoenix dactylifera* L.) ونخيل واشنطن (*Washingtonia filifera*) (Linden ex André) H. Wendl. ex de Bary الناجم عن التعرض للأشعة فوق البنفسجية نوع –ب (UV–B) على فترات مختلفة هي(2 و 4 و 8 و 10 ساعه/ يوم). أظهرت النتائج أن 30 يومًا من المعاملة بالأشعة فوق البنفسجية نوع–ب لمدة 2 و 4 تسببت في زيادة كبيرة في إجمالي محتوى الكلوروفيل لكلا النوعين من شتلات النخيل بينما تم الحصول على أقل محتوى من الكلوروفيل في النباتات المزروعة تحت ضغط الأشعة فوق البنفسجية – ب لمدة 10 ساعات / يوم . وعلى العكس ، تم تسجيل أعلى قيمة لمحتوى الكاروتينوات عند 8 ساعات / يوم من المعاملة بالأشعة فوق البنفسجية ، ثم انخفض عند المعامله بالـ 10 ساعات / يوم . وبالمثل ، أظهر محتوى الفينولات الكلي والبرولين زيادة كبيرة مع زيادة وقت التعرض للأشعة فوق البنفسجية ب في كلا النوعين . وكشفت الدراسة أيضًا عن وجود اختلاف كبير بين النوعين من النخيل من ناحية التحمل للأشعة فوق البنفسجية – ب ، حيث وجد أن شتلات نخيل البلح (*Phoenix dactylifera* L.) أكثر مقاومة لمثل هذا الإشعاع من (*Washingtonia filifera*).

Introduction

Plants use the sunlight for photosynthesis, and thus they exposed directly to different spectra of ultraviolet radiation (UV) that are reaching the earth's planet from the sunlight. In general, UV Light divide into three types according to wavelength, these are UV- A (320-400 nm), UV –B (280–320 nm) and UV-C (100-280nm). Fortunately, the UV-C completely absorbed by the layer of ozone in the stratosphere, while, some of the UV-B rays penetrate the atmosphere towards the earth. (Caldwell *et al*., 1989).

Exposure of plants to UV radiation may cause physiological changes like growth inhibition, increasing phenolic compounds and reduction in Rubisco activity due to scaling down the photosynthetic genes. However, plants have developed several mechanisms to save their systems from harmful radiations. One of the most important of these mechanisms is the UV-absorbing compounds (Physiological parameters).

These factors are used as an effective indicator to determine the sensitive and tolerant plant to the detrimental UV-B radiation (Stapelon, 1992; Sharma *et al*., 1998; Hollosy, 2002; Brzezinska *et al*., 2006; Shaukat, *et al*., 2013). Accordingly, Tegelberg (2002) found that the flavonoids absorbing substances like phenolic acid increased in silver birch leaves when exposed to the high level of UV-B radiations.

In date palm, Al-Enezi and Al-Khayri (2012) study changes of physiological and biochemical aspects initiated by X-radiations using different X-rays doses .the researchers refer that photosynthetic pigments content reduced with dose increases. Whereas, Niazwali (2016) studied the impact of UV-B radiation on UV-absorbing compounds in date palm seedlings. He found that phenols and proline were accumulated, but total chlorophyll and carotenoids were reduced as a result of UV treatment. Thus, due to the limit information about the assessment of defence system in palm species against the UV- radiation, therefore we evaluate in the present investigation the effect of different doses of UV- B on two palm species under laboratory conditions.

Materials and Methods

The experiment was conducted in the Department of Biology, University of Basrah, Basrah, Iraq during the year 2017-2018. Seeds of two palm species, (*Phoenix dactylifera*) cv. Barhii and (*Washingtonian filifera*) were collected on summer season from an orchard located in Uosffan, Aboalkhasieb South East of Basrah Province and the Garden of Science Collage respectively. All selected seeds washed in tap water for 15 minutes, then sterilized with 20% sodium hypochlorite for 20 minutes, followed by washed 3 times with sterilized distilled water. Sterilized seeds were then soaked in distilled water in a plastic tray under room temperature for 72 hours before sowing in pots. Following, seeds were sown individually in plastic pots sized (12×10.8×8.8cm), containing a mixture of clay, peat moss and vermiculite (1:1:1 v:v). Pots were stored in a growth chamber at 30°C and 50-60% relative humidity. Palm seedlings were irrigated every 2-3 days or when the plant needs. Plants were fertilized with half power of Hogland solution and with rate one day/week.

Identical and healthy seedlings at 6 weeks old were used to perform this experiment.

UV-Treatments

One emission tube of UV- B tube (30w, λ 280-320 nm) was fixed between normal white florescent lights on the top of the wooden chamber. Supplementary florescent source lights with a rate of 75 μmol m–2s–1 provided by using 5 fluorescent tubes and photoperiod 12-16 hours/day. The distance between the light source and plants reached 80 cm. To avoid plant tropism, the walls of the wooden cabinet were covered with reflective paper to ensure the delivery of the light in different directions.

The similar healthy germinated seedling of both palm species divided into 5 groups of 10 pots. UV-B exposure dose was given to 5 groups with different rates as follows: 0, 2, 4, 8 and 10 hours/ daily. Programmable digital timer type TS-EE8 was

used to control the daily UV-exposure time for each group. All Palm plants grown under artificial light without UV source were considering as a control treatment. For studying the physiological parameters, plant samples were taken after 30 days of grown under UV-B stress.

Estimation of Chlorophyll and Carotenoids Contents

The chlorophyll and carotenoids were extracted from the palm leaves according to the method described by Horwitz (1975). The total chlorophyll and carotenoids were calculated by the following equations:

Total chlorophyll ($Mg.L^{-1}$) = 20.2 × Ab. (645) + 8.02 × Ab. (663)

Total Carotenoids ($Mg.L^{-1}$) $= \frac{EY}{e100} \times$ 1000 mg

E: Absorbance at 480nm.

Y: final volume after dilution with acetone.

e: carotenoid constant 2300.

$Mg.L^{-1}$unit turned to $Mg.L^{-1}$/100g unit as the following formula of Zaehringer et al, (1974).

$$mg/100g = \frac{100}{sample\ wei1ght\ (g)} \times \frac{mg/L}{1000}$$

Estimation of Total Phenols Content

One gram of the dry plant tissues was mixed with 8 ml of distilled water and kept in the water bath at 70◦c for 1 hour. The mixture then left until cooled and filtrated. After that, 1.5ml of diluted FolinCiocalteau reagent was added to 1ml of the supernatant. After 5 minutes, 1.5 ml of a 6% sodium carbonate solution was added. Finally, the absorbance of phenols was determined at 725 nm. Total phenol was estimated by using the standard curve prepared with different concentrations of titanic acid, the results were expressed by $Mg.100gm^{-1}$ dry weight according to Melo, *et al*., (2005).

Estimation of Proline Content

0.2 g of the dried leaf was ground to make it a powder. The plant sample was extracted with 5 ml of 95% ethanol. The extracts were centrifuged at 1600 rpm for 10 minutes. After that, the Supernatant was evaporated completely until dryness. The residual mixture was dissolved in 2 ml of distilled water and centrifuged again at 1600 rpm for 10 minutes. The Absorbance was determined at 520 nm. The proline amount was calculated from the standard curve prepared with proline. Proline contents were expressed in $Mg.100gm^{-1}$ according to Troll and Lindesly (1955).

Statistical Analysis

The experiment was randomly designed with two factors. Results were analyzed using the analysis of variance (ANOVA). And the means were separated, by

using the least significant difference (LSD) at 5%. Data represent the means of three replicas for each treatment.

Results and **Discussion**

Effect of UV-B radiations on Total Chlorophyll and Carotenoids Content

The exposure of palm seedlings of both species to UV-B radiation caused changes in total chlorophyll content. In date palm (*Phoenix dactylifera*) the total chlorophyll content was increased in plants grown under UV- light treatments for (2, 4 and 8 hours), where the chlorophyll content recorded (24.02, 22.23 and 22.18 Mg.100gm^{-1}) respectively. Whereas, (*Washingtonia filifera*) demonstrated high chlorophyll content only, when plants exposed to UV-B radiation for 2 and 4 hours. Following, the total chlorophyll content dropped to15.50 and 14.89 Mg.100gm^{-1}, when UV dose increased to (8 and 10 hours) respectively (Table-1 and Fig-2). Moreover, the carotenoids pigments increased also when seedlings of both species subjected to UV- radiation for (2, 4, 8, and 10 hrs.). While, the results of analysis for carotenoids of *both species* recorded (0.183, 0.203, 0.195, 0.191,0.140, 0.160,0.158 and 0.150 Mg.100gm^{-1}) respectively (Table-2) and (Fig-3).

Effect of UV-B light on total phenol and Proline

The observations in the present study revealed that the number of phenolic compounds was increased significantly in leaves of (*Washingtonian filifera*) when the seedling has grown under UV-B light for 2 and 4 hours per day. Where, it reached to 1.00 and 1.027 Mg.100gm^{-1}, (Table-3. and Fig-4). But it was suddenly dropped to 0.850 and 0.820 when the plants exposed to UV-B radiation for a long time (8 and 10 hours/ day) respectively. In date palm (*Phoenix dactylifera*), the level of the absorbance phenolic compounds also increased, but it semi-stable for all treatments (Fig-4, Table-3). Thus, these results reflex the strong correlation between the accumulations of phenolic compounds and UV-B tolerance between the two species of palms. On the other hand, the present study showed that distinctly high proline amounts (25.09 and 27.09) were recorded in date palm (*Phoenix dactylifera*) seedlings exposed to UV- light for a prolonged period (8 and 10 hours). Whereas, in (*Washingtonian filifera*) level of proline in the seedlings increased to 14.1 and 17.91 Mg.100gm^{-1} when it the extent of UV-B treatments increased to 4 and 8 hours (Table-4 and Fig-5). But, extended the exposition of UV-B light to 10 hours per day led to drop the proline content to 9.46 Mg.100gm^{-1}. Therefore, these results give a strong indicator to the ability of the defence system in date palm seedling to stand up against the UV-B radiation.

Some UV light spectrums are important to life, where it plays a vital role in the control of the plant pathogenic microorganism. But unfortunately, the short wavelengths of this radiation ranging between 280-200nm (UV-B and UV-C) have a deleterious impact on various organisms and ecosystems (Teramura, 1983, Bornman *et al*., 2015; Rai and Agrawal, 2017). However, plants have been evolutes their defence systems to stand up against such radiations. And Scientists have demonstrated the

various mechanisms that help the plant to protect itself from the harmful electromagnetic radiation. Generally, the defence system divided into two mechanisms, these are: The first defend line is regarding the structural modification of the organ system through cell wall thickening and wax cuticle formation. While, the second defend line represented by the accumulation of carotenoids, proline, flavonoids, anthocyanins or other UV absorbing compounds in the leaf epidermis (Singh *et al*., 2008; Salama *et al*., 2011; Moghadam *et al*., 2012). So, the terrestrial plants follow a special distribution on lower latitudes or higher elevations, through their ability to resist the high levels of UV-B (Turunen and Latola, 2005).

In whatever way, present results revealed the potential of date palm (*Phoenix dactylifera*) to stand up averse to prolonged exposure to UV-B in compared with (*Washingtonian filifera*) seedlings. Whereas, the total chlorophyll content in date palm leaves showed less marginal reduction (Table-1). This result is in the line with Niazwali, (2016) who also found slight scaling down in total chlorophyll content of treated plants with UV- B for 4 and 8 hrs. On the other hand, exposure the (*Washingtonian filifera*) seedlings to UV-B for long period (8 and 10 hrs.) caused a substantial reduction in chlorophyll (Tabl-1 and Fig-2). Same results were obtained when the almond plant (*Prunus dulcis*) put through UV-B, where it caused a considerable reduction in the content of both Chlorophyll (a) and (b). Jordan et al,(1994) reported an increased level of UV radiation may cause oxidative damage in chlorophylls and polyunsaturated lipids through the formation of free radicals peroxides. While Jansen *et al.*, (1998) and Sullivan and Rozema, (1999), proposed that the changes in total chlorophyll content reflect the possible damage of the ultra-structure of chloroplast and changes in photosynthetic pigments. But, Mackerness *and* Thomas, (1999) suggested that plants grown under UV-B stress may sacrifice the chloroplast to save the rest of the cell. But, Jacobs *et al*., (2007) believed that plants can able to survive under UV-stress condition may evolve their capacity to scavenging the free radicals. However, we must keep in mind that all these explanations mentioned above definitely depend on the plant's species and development stage. On the other hand, analyze leaf carotenoids give rise good evidence for the efficiency of the defence system to protect the plant from the UV-B radiation. In the present investigation, it was found that the level of carotenoids was increased when date palm seedlings were subjected to a high dose of UV-B (8-10 hrs.). Al-Enezi and Khayari (2012) who found a hike in carotenoids when date palm seedlings were exposed to X-radiations obtained the same results. On the contrary, (*Washingtonian filifera*) seedlings showed a reduction in carotenoids level when UV dose increased to 8-10 hrs (Fig-3). Phenols compounds are another defence line that increases plant resistance against UV-B radiation, desiccation and freezing (Chalker-Scott, 1999; Hoch *et al*., 2001; Teleberg *et al*., 2002). In this study, the date palm plants exhibited substantially higher contents of phenolic compounds after enhanced to UV-B (8-10 hrs./ day) as compared with (*Washingtonian filifera*) Fig-. This result is agreed with the Niazwali, (2016), who also found increasing of phenols in notable amount after exposing the seedlings to UV- light for 4 and 8 hrs. /day.

In the present study, we also found that the UV-B was stimulated the proline accumulation in date palm seedling after (2-10 hrs. daily exposure). Whereas, in (*Washingtonian filifera*) seedlings the proline accumulation dropped after (10 hrs. daily exposure to UV-B) Fig-4. This result is in the line with Niazwali, (2016), who also observed the increase in proline amount of different date palm varieties treated with 4 and 8 hrs. of UV-B. Al-Enezi and Al-Khayri, (2012) also obtained the same results when date palm seedlings were irradiated to X-rays.

Because the proline has an ability to scavenge the free radical promoted by abiotic stress (Fedina *et al*. 2002). So this study concluded that the proline may have an important role to make the date palm seedlings resisted the high dose of UV-B radiation.

Table 1:Effect of different UV-B doses on total chlorophyll content (Mg.100gm^{-1}**)**

Plant Species	Daily Exposure Time (hr.)					Effect of species
	0	2	4	8	10	
	Total Chlorophyll Content(Mg.100gm^{-1})					
Phoenix dactylifera	19.90 ^{cd}	24.02 ^{a}	22.23^{b}	22.18^{b}	19.65^{cd}	21.60^{a}
Washingtonian filifira	18.38^{d}	20.47^{c}	19.56^{cd}	15.50^{e}	14.89^{e}	17.76^{b}
Effect of Exposure Time	19.14^{c}	22.25^{a}	20.89^{b}	18.84^{c}	17.27^{e}	
l.s.d at 5% level						
Species: 0.70		Exposure Time: 1.11			Species and Exposure Time: 1.57	

Values followed by the different letter within the same group indicate a statistically significant difference

Table 2: Effect of different UV-B doses on carotenoids content (Mg.100gm^{-1}**)**

Plant Species	Daily exposure time (hr.)					Effect of Species
	0	2	4	8	10	
	Total Carotenoids Content (Mg.100gm^{-1})					
Phoenix dactylifera	0.153^{cd}	0.183^{ab}	0.203^{a}	0.195^{a}	0.191^{a}	0.185^{a}
Washingtonian filifira	0.130^{d}	0.140^{cd}	0.160^{bc}	0.158^{c}	0.150^{c}	0.148^{b}
Effect of Exposure Time	0.142^{c}	0.162^{b}	0.182^{a}	0.176^{ab}	0.170^{b}	
l.s.d at 5% level						
Species: 0.011		Exposure Time: 0.017			Species and Exposure Time: 0.024	

Values followed by the different letter within the same group indicate a statistically significant difference.

Table 3: Effect of UV-B doses on phenols content (Mg.100gm^{-1})

Plant Species	Daily Exposure Time for UV-B (hr./day)	Effect of Species

	0	2	4	8	10	
	Total Phenol content (mg/100g)					
Phoenix dactylifera	0.600^{f}	0.670^{e}	0.770^{d}	0.800^{c}	0.817^{e}	0.731^{b}
Washingtonian filifira	0.890^{c}	1.000^{a}	1.027^{b}	0.850^{d}	0.820^{e}	0.917^{a}
Effect of Exposure Time	0.745^{d}	0.835^{b}	0.898^{a}	0.825^{c}	0.818^{c}	
l.s.d at 5% level						
Species:0.005		Exposure Time: 0.008			Species and Exposure Time: 0.011	

Values followed by the different letter within the same group indicate a statistically significant difference.

Table 4: Effect of UV-B doses on proline content (Mg.100gm^{-1})

Plant Species	Daily Exposure Time (hr./day)					Effect of Species
	0	2	4	8	10	
	Total Phenol Content (Mg.100gm^{-1})					
Phoenix dactylifera	5.52^{f}	9.59^{e}	17.59^{c}	25.09^{b}	27.09^{a}	16.98^{a}
Washingtonian filifira	4.26^{g}	9.26^{e}	14.01^{d}	17.9^{c}	9.46^{e}	11.00^{b}
Effect of Exposure Time	4.89^{e}	9.43^{d}	15.85^{c}	21.50^{a}	18.28^{b}	
l.s.d at 5% level						
Species: 0.35		Exposure Time: 0.55			Species and Exposure Time:0.76	

Values followed by the different letter within the same group indicate a statistically significant difference.

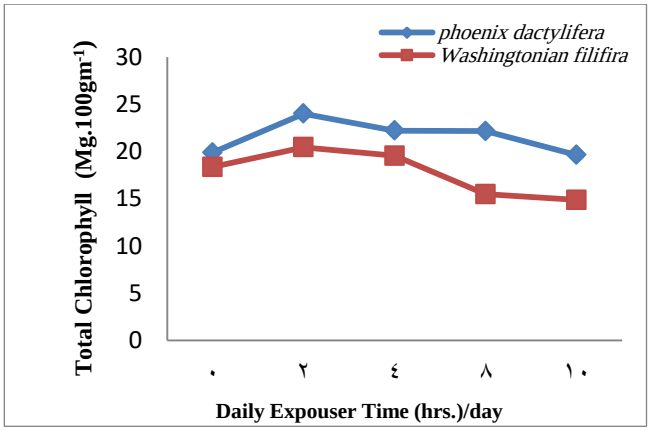

Fig-1 The influence of UV-B radiation on chlorophyll content

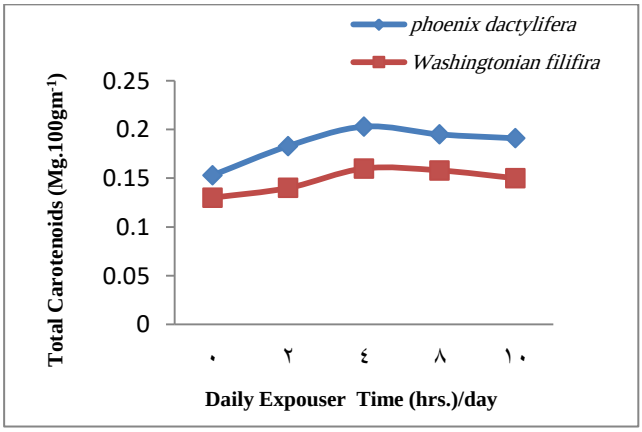

Fig-2 The effect of UV-B radiation on carotenoids content

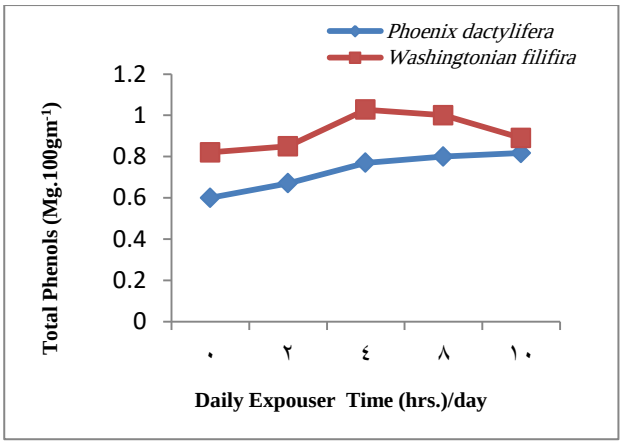

Fig-3 The impact of UV-B radiation on phenols content.

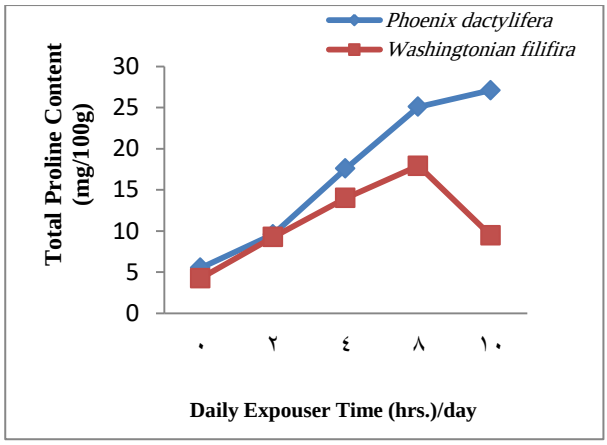

Fig-4 The influence of UV-B radiation on proline content.

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