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ORIGINAL ARTICLE

Responses of *Carthamus tinctorius* at Two Development Stages to Low Light Intensity (LLI): Changes on Phenolic Metabolites and Related Antioxidant Activities

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We investigated the contributions of low light intensity (LLI) and development stage on growth status, nutrient uptake, pigment contents, bioactive molecule contents and biological activities on *carthamus tinctorius* plants at two growth periods: vegetative and flowering periods in order to optimize natural culture conditions required to improve leaf antioxidant accumulation; while maintaining acceptable biomass production under LL conditions. For this purpose, an open field culture experiment was conducted on safflower leaves subjected to optimal light (250 µmol m^{-2} s^{-1}) or LLI condition (125 µmol m^{-2} s^{-1}). Shade constraint affected extensively the growth in terms of dry weight, RGR and water content particularly at vegetative stage. MDA and EL levels had a noteworthy increase particularly at flowering stage S reaching +34%in comparison to sun-exposed leaves. In addition TPC, TFC and TCT were mostly enhanced at full flowering stage than vegetative one. In the same line, the antioxidant activities were found to be enhanced at the FS stage as compared to the vegetative one. These results strongly indicate that LL induces the accumulation of secondary metabolites in *C. tinctorius* leaves by altering the phenolic synthesis pools, as well as for the up-regulation of antioxidant molecules defense

Key words: Low light intensity, vegetative stage, floral stage, phenolic pools, antioxidant activities

Light is a major environmental factor that plays an important role in plant development and metabolism (Berenschot and Quecini, 2014). In fact, light is crucial for photosynthesis and photo-morphogenesis. Low light (LL) constraint, as abiotic stress, can result from light blocking by horticulture facilities, clouds and eventually snow. It was shown to substantially affect plant agronomic traits and hinders physiological metabolic processes, including photosynthesis and antioxidant production, as well as carbon and nitrogen fixation (Zhu et *al.*, 2017). LL stress usually inhibits plant growth and productivity by affecting gas exchange (Zhan et *al.*, 2002). In fact, it affects negatively leaf thikness and stomatal size (Huang et *al.*, 2004; Wei et *al.*, 2005; Gregoriou et *al.*, 2007), Many studies showed that light stress was dependent on plant developmental stage (Deng et *al.*, 2009; Liu et *al.*, 2009).

The chloroplast lumen in plants at LL becomes acidic in nature, and excitation energy accumulates within chloroplasts; which results in generation of singlet and triplet forms of singlet oxygen Due to the excess light absorption, according to Ali et al. (2005),. This will be accompanied by ROS generation such as superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2).Low light causes oxidative stress in plants and induces ROS accumulation. In general, ROS over generation harm cell membranes and functions (Zhang et *al.*, 2011).

In order to alleviate ROS induced oxidative damage, plants induce different mechanisms blocking ROS-generating chain reactions propagation (Jelali et *al.*, 2017). Polyphenols are among the most active secondary metabolites implicated in response to low light intensity. These metabolites are considered potential molecules owing to their chemical structure. Indeed, due to their particular basic structure they exhibit important antioxidant properties (Huang et *al.*, 2005; Wasli et *al.*, 2018).These bioactive metabolites are synthesized in different plant parts and contribute in preventing ROS accumulation) at toxic levels during abiotic/biotic stresses (Naczk and Shahidi, 2006). In plants, polyphenol biosynthesis and accumulation are generally stimulated in response to biotic/abiotic constraints (Naczk and Shahidi, 2004). Thus, shade-stressed plants might represent potential sources of polyphenols, by increasing tissues polyphenol accumulation. Optimal polyphenol yield would be obtained using stress-tolerant species (Bettaieb et *al.*, 2012)

Tunisia has a wide array ofaromatic and medicinal plants used mainly for culinary and for therapeutic purposes, thus representing potential sources of active biomolecules. Among them, *carthamus tinctorius*i safflower (Asteraceae family), is well cultivated crop in Mediterranean regions, Europe and central Southern Asia and frequently used for flavoring and seasoning meals(Karray-Bouraoui et *al.*, 2011; Zaoui et *al.*, 2016).Safflower is actually cultivated for its edible oil and as a birdseed (Gyulai 1996). This oriental herb is cultivated mainly in arid and semi-arid environments due to its ability to tolerate abiotic stresses this criterion is linked to its rich pool of antioxidants, including phenolic compounds which are important in protecting biological molecules against oxidative damage (Karray-Bouraoui et *al.*, 2010; Karray-Bouraoui et *al.*, 2011).

Safflower responses to salinity and drought along with other abiotic stress have been intensively studied using physiological, biochemical and metabolic traits (Ben Abdallah et *al.*, 2013; Salem et *al.*, 2014; Zaoui et *al.*, 2016) but data relative to its secondary metabolism under low light intensity particularly in open-field conditions still scarce. For this reason, the present study was carried out to assess shade condition effects on growth activity, photosynthetic performance and production of phenolic compounds in *Carthamus tinctorius* L., this study was conducted firstly to define culture conditions optimizing biomass and biomolecules production and secondly to estimate polyphenols and antioxidant activity variations depending in leaf stage. This study will be useful for crop breeders and growers to produce safflower with high levels of natural antioxidants even in shade conditions. These findings would be a useful tool in determining plant growth requirements for quality enhancement of medicinal plants.

MATERIALS AND METHODS

Plant culture, growth conditions and harvest

Experimental fields were conducted in Djedeida provenance 36°51 '02 "Nord 9° 56' 10" East, Tunisia (Figure 1); which is characterised by a semi-arid climate with mean annual rainfall ranging from 0.4 to 87 mm. The soil type at the experimental site was loam, classified as Typic Haplustalfs Machacha (2011), with a pH of 7.2, and electrical conductivity (EC) of 442 µS/cm. In the field, the soil was basally dressed using a commercial fertiliser (NPK ratio of 2:3:2 (22%)) at 19.8 kg/ha N. After germination, plants were watered daily at dawn until the 4th true leaf stage, to maintain soil moisture near field capacity until the soil surface underneath the plants was covered with water for approximately 2 s.

Seeds were sown in soil and then irrigated for 60 days with distilled water until germination was occurred. Seedlings were watered daily with a nutrient solution of Hoagland and Arnon (1950)[1.25 mm Ca $(NO_3)_2$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, 0.25 mM KH_2PO_4 and 10 µm H_3BO_3, 1 µM $MnSO_4$, 0.5 µm $ZnSO_4$, 0.05 µm $(NH_4)_6Mo_7O_{24}$ and 0.4 µM $CuSO_4$]. Safflower plants were grown either in shade conditions by disposing the plants on a sloping slope whichretains50% of light (shade treatment) or exposed to direct sunlight (control treatment). During experimental period light intensity was measured with a quantum sensor light meter with separate sensor (QMSS) from germination to harvesting for data analysis. Each reading was expressed as µmol m^{-2} s^{-1}.*C. tinctorius* leaves were harvested at two growth stages: vegetative stage and full-flowering stage (Figure 2). Twenty-five plants were harvested for every growth stage; only leaves were used in experiments and divided to three groups: the first was dried and used for growth parameters evaluation, the second was kept fresh and used for biochemical parameters and the third was dried and used for polyphenols extraction and determination.

Plant growth evaluation and mineral analysis

After 3 days of oven drying at 60° C, leaves dry weight (DW) was determinate, and their water content was calculated as (FW - DW)/FW, where FW and DW represent the fresh and dry weight, respectively. Relative growth rate (RGR) values, based on leaf dry weight, were determined as follow: (RGR, day^{-1}) = ln W_2- ln W_1/105 or ln W_2 - ln W_1/135), whereW_1andW_2aredry weights at the beginning and the end of the treatment period, and 105and 135are the treatment duration in days. An amount of 20 mg from grounded oven-dried leaves at both growth stages was digested with 25 mL of nitric acid solution (HNO_3 0.5 %). After 15 days, the mixtures were filtered through a Whattman filter paper, and K^+ and Ca^{2+} concentrations in the digests were determined with a flame emission photometry (Jenway PFP7) after calibration with standard solutions (Karray-Bouraoui et *al.,* 2010).

Measurement of photosynthetic parameters

The variation of pigment concentrations was assessed by measuring absorption spectra of frond extracts using a UV spectrophotometer (Spectro.UV-VIS Dual Beam 8 Auto cell UUS-2700). Two hundred (200) mg of safflower plant material frozen in liquid N_2 was ground to a fine powder (on ice) and immediately immersed in 5 mL of 80% acetone solution. Then the total extraction took place after 72 hrs in darkness, at 4°C and absorbance of extracts was measured in the supernatants after 3 days at 663, 645 and 470 nm respectively for ChLa, ChlL b and carotenoids (Lichtenthaler and Buschmann, 2001).

Lipid peroxidation and electrolyte leakage

The level of lipid peroxidation was measured as 2-thiobarbituric acid-reactive substances (mainly malondialdehyde (MDA) according to (Jelali et *al.,* 2014). Frozen samples (1g of fresh material) were homogenized with a pre-chilled mortar and pestle with 10 mL of (0, 1%; p/v) trichloroacetic acid and centrifuged at 10000 g for 10 min and at 25°C. 1ml of the upper liquid layer (supernatant) was added to 4 ml thiobarbituric acid (TBA) (0,5 %; p/v). After centrifugation at 1000 g for 10 min, the supernatant absorbance was read (532 nm) and values corresponding to non specific absorption (600 nm) were subtracted. MDA concentration was calculated using its molar extinction coefficient (155 $mM^{-1}{\cdot}cm^{-1}$) according to the following formula:

$$MDA\ (nm/\ gFW) = [(OD_{532} - OD_{600})*vs/0.155] \times FW$$

Electrolyte leakage parameter was determined on excised leaves of safflower (Mao et *al.,* 2007). After being rinsed for 2–3 min with deionized water, 5 pieces

were immersed in 20 mL of deionized water in test tubes and shaken every 5 min for 30 min.. Total conductivity was accoured after keeping the test tubes boiling for 15 min ; then results was expressed as percentage of total conductivity.

Determination of total phenolic contents (TPC)

The total phenolic content (TPh) of safflower extract from each treatment was assessed using a colorimetric assay based on the Folin-Ciocalteu reagent. A volume of 125 µL of SE was added to 60 µL of H_2O and 15 µL of the Folin-Ciocalteu reagent (0.2 mol. L^{-1}). After shaking, 150 µL of Na_2CO_3 (7%) was added. After incubation for 1 hour at room temperature, the optical density (OD) at 750 nm was determined. Results were expressed as mg of gallic acid equivalent per g of dry weight (GAE g^{-1} DW) using a calibration curve (Dewanto et *al.,* 2002).

Determination of total flavonoid contents (TFC)

For total flavonoid content (TFC), 250 µL of safflower extract was mixed with 75 µL $NaNO_2$ (5%; w/v). Then, 150 µL of $AlCl_3/6H_2O$ (10%; w/v) and 500 µL of NaOH (1 M) were added after 6 min of incubation. After adjusting the volume to 2500 µL with H_2O, the absorbance was determined at 510 nm. TFC were expressed as mg (+)-catechin equivalent/g DW (mg CE. g^{-1} DW) (Dewanto et *al.,* 2002).

Determination of condensed tannins contents (TCT)

Proanthocyanidin were revealed according to vanillin-H_2SO_4 method (Saada et *al.,* 2014). Fifty µL of safflower extract from each treatment were pipetted out into a test tube. Then, 3 mL of 4% methanolic-vanillin solution and 1.5mL of concentrated H_2SO_4 were added and vortexed. Tubes were stand for 15 min. The absorbance was measured at 500 nm. The amount of condensed tannins were expressed as mg catechin equivalent/ g DW (mg CE. g^{-1} DW).

Total antioxidant capacity (TAC)

Total antioxidant capacity was assessed according (Saada et *al.,* 2014). Briefly, 100 µL of SE was combined to 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was allowed to cool, after incubation at 95°C for 90 min. The absorbance was measured at 695 nm and TAC was expressed as mg gallic acid equivalent / g DW (mg GAE. g^{-1} DW).

ABTS scavenging activity

Antiradical capacity of safflower extract (SE) against ABTS radical was assessed according to Wasli et *al.* (2018) method. Briefly, 250 µL of stable radical ABTS solution (prepared by reacting the stock solution of ABTS (7 mM) with potassium persulfate (2.45 mM) in a ratio of 1:1) was added to 50 µL of increasing concentrations of SE. After 6 min of incubation at room temperature, the absorbance was read against a blank at 734 nm using an ELX800 microplate reader. ABTS scavenging ability was expressed as IC_{50} (mg/ mL) which is the inhibiting concentration of 50% of the synthetic radical.

The inhibition percentage (IP %) of ABTS radical was calculated using the following formula:

$$IP\ (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Iron reducing power

Ferric reducing antioxidant power (FRAP) was focused on the reduction of the trivalent iron produced by the $FeCl_3$ (Wasli et *al.,* 2018). The intensity of the blue-green color was measured at 700 nm. Values were expressed as EC_{50} (mg/mL): the effective concentration of SE corresponding to an OD = 0.5.

β-carotene linoleic acid model system

Initially, *β*-carotene (20 mg) was suspended in 10 mL chloroform; linoleic acid (50 mg) and Tween 80 (1g) were added to 1 mL of this solution (Wasli et *al.,* 2018). Chloroform was removed using a vacuum at 40°C, and 100 mL oxygenated water added.The obtained β-carotene/linoleic acid emulsion was vigorously shaken before 250 µL was added to each well of 96-well microliter plates alongwith 50 µL of test samples.The initial absorbance at 470 nm was recorded.

The emulsion system with two controls [one containing BHA as a positive control (Figure 1) andthe other with the same volume of distilled water instead of the extracts]was incubated at 50C for 120 min and the absorbance at 470 nmread using a model ELX800 microplate reader. Readings for all samples were performed immediately and after 120 min of incubation. The antioxidant activity of the extracts was evaluated in

terms of blanching inhibition of the β-carotene as follows:

$$\%\ of\ inhibition\ = \frac{((C_{t=0} - C_{t=2}) - (E_{t=0} - E_{t=2})}{(C_{t=0} - C_{t=2})} \times 100$$

Statistical analyses

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test was performed. The statistical tests were applied using Graph Pad Prism, version 6 and the significance level was $p < 0.05$. Multivariate data analysis was carried out using principal component analysis (PCA). The PCA type used is Pearson's correlation and it was done using XLSTAT, considering variables centered on their means and normalized with a standard deviation of 1.

RESULTS

Plant growth parameters

The effects of LLI and growth phases on dry weight, hydration and relative growth rate are shown in Table 1. Results showed a clear depressive effect of shade condition on leaf dry weight (DW) for both development stages; with a higher effect in vegetative than flowering stage. Indeed, during the vegetative period the decrease, was about -94%; contrawise, -80% during the reproductive one as compared to the control. Relative growth rate, exhibited also the same pattern observed for (DW) where the reduction was about -44% and -25% respectively in vegetative and flowering stages. As well, a relevant decline in water content was occurred in response to LLI with a more pronounced effect in floral stage (-27%) than the vegetative period (-18%) (Table 1).

Potassium (K^+) and calcium (Ca^{2+}) contents; were distinctly reduced in flowering periods (-29% and -24% as compared to control) with a slight decrease at vegetative stage (Table 1).

Photosynthetic pigments

For both physiological stages, LLI had a regressed effect on leaf chlorophyll contents. In fact, as compared to control plants, a reduction in ChL *a*, ChL *b* and T ChL contents was noted predominantly in flowering stage (-66%, -33% and -60% and -24% respectively) as compared to control leaves (Table 1).

Lipid peroxidation and electrolytes leakage

Shade conditions had a noteworthy effect on leaf MDA content in *C.tinctorius* leaves during vegetative and flowering stage; where the relevant increase was especially detected at full flowering stage (+34%) in comparison to sun-exposed leaves. Similarly, electrolytes leakage was significantly correlated with lipid peroxidation results (Table 2). Indeed, EL was incremented at both periods, and was more superior in flowering period than in the vegetative one as compared to the normal plants.

Change in phenolic pools as function of physiological development stage

Results of quantitative estimation of total phenols, flavonoïd and tannins contents of *C. tinctorius* leaves during different growth stages and LLI conditions are presented in Table 3. LLI induced a noteworthy accumulation of polyphenols at both physiological stages predominantly in floral stage; where it increased in twice from 19 mg/g GAE DW) (shaded leaves) to (41 mg GAE/g DW). With similar tendency to phenol, flavonoid content was remarkably higher in shadingplants; where the highest amounts waere detected during the flowering period (23.74 mg GAE/g DW of the plant. Concerning the condensed tannins, these compounds seem were also incremented at both periods (from 13.61 to 16.50 and from 15.12 to 21.44 respectively for vegetative and flowering stage) (Table 2).

Change in antioxidant as function of physiological development stage

TAA of the two developmental stages was extremely different (Figure 3). Indeed, this ability was 1.5-fold higher in the flowering stage (45 mg GAE/g DW) than that in vegetative one (29.7 GAE/g DW). The exposure to LLI, the antioxidant activity increased in the leaves of *C. tinctorius* and was in proportion with the total phenols. The magnitude of ABTS· radicals quenching activity seemed to be related to the physiological stage and LLI too, as IC_{50} values largely differed between the two periods (Figure 3). The highest antiradical activity against ABTS was recorded for the plants exposed to LLI at both stages and it reached 411 µg/mL (vegetative period) and 207 µg/mL(flowering period) The trend for

ferric reducing activity of the different growth periods showed a similar tendency as compared to their ABTS radical scavenging activities, when a comparison between EC_{50} and IC_{50} propensities is made. In fact, flowering stage showed relatively the stronger FRAP activity reflected by low EC_{50} values, as compared to the vegetative stage ones. For β-carotene/linoleate system, shoots displayed the highest capacity of inhibiting the lipid peroxides as compared to different treatment with the lowest IC_{50} value (3.7 mg/mL) from floral stage.

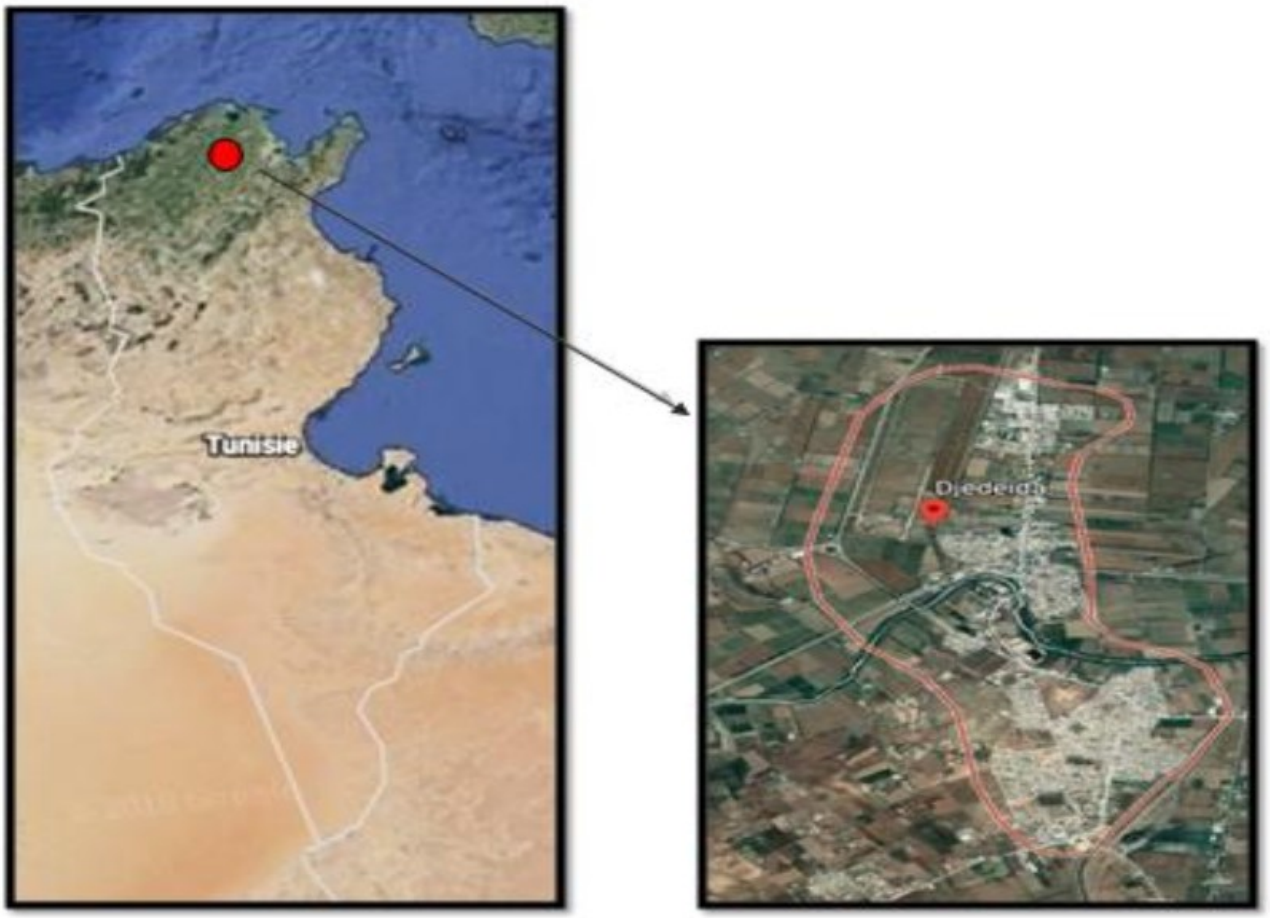

Figure 1. Geographical localization of Djedeida provenance (Governorate of Manouba).

Figure 2. *Carthamus tinctorius* at vegetative (A) and floral (B) periods

Table 1. Effects of LL treatment on growth parameters, photosynthetic pigments and nutrient uptake in leaves of *C. tinctorius* plants. Values are means of six replicates and standard deviation. Values with different superscripts (a-d) are significantly different at P < 0.05.

	FLI		LLI	
	Vegetative period	Flowering period	Vegetative period	Flowering period
DW (g/ plant)	1.37±0.240^{A}	0.30±0.01^{B}	0.078±0.01^{C}	0.059±0.01^{D}
RGR (day^{-1})	0.19 ± 0.024^{A}	0.094±0.07^{B}	0.008 ± 0.00^{C}	0.006 ± 0.002^{D}
Water content (mL/ g DW)	3.58±0.019^{A}	2.93±0.34^{B}	2.71±0.89b^{C}	1.98±0.03^{D}
K^+ (mg/g DW)	1.25±0.45 ^{A}	1.19±0.21 ^{A}	1.13±0.19 ^{A}	0.85±0.29 ^{A}
Ca^{2+} (mg/g DW)	0.98±0.11 ^{A}	0.76±0.09 ^{A}	0.85±0.05 ^{A}	0.58±0.17 ^{A}
ChL *a* (mg/g FW)	0.79±0.05^{A}	0.67±0.02^{B}	0.36±0.06^{C}	0.23±0.03^{D}
ChL *b* (mg/g FW)	0.21±0.04^{A}	0.15±0.09^{B}	0.15±0.02^{C}	0.10±0.00^{D}
ChL *a* +*b*(mg/g FW)	1.00±0.09^{A}	0.82±0.04^{B}	0.51±0.08^{C}	0.33±0.04^{D}
CAR (mg/g FW)	0.125±0.01^{A}	0.133±0.01^{B}	0.157±0.12^{C}	0.187±0.09^{D}

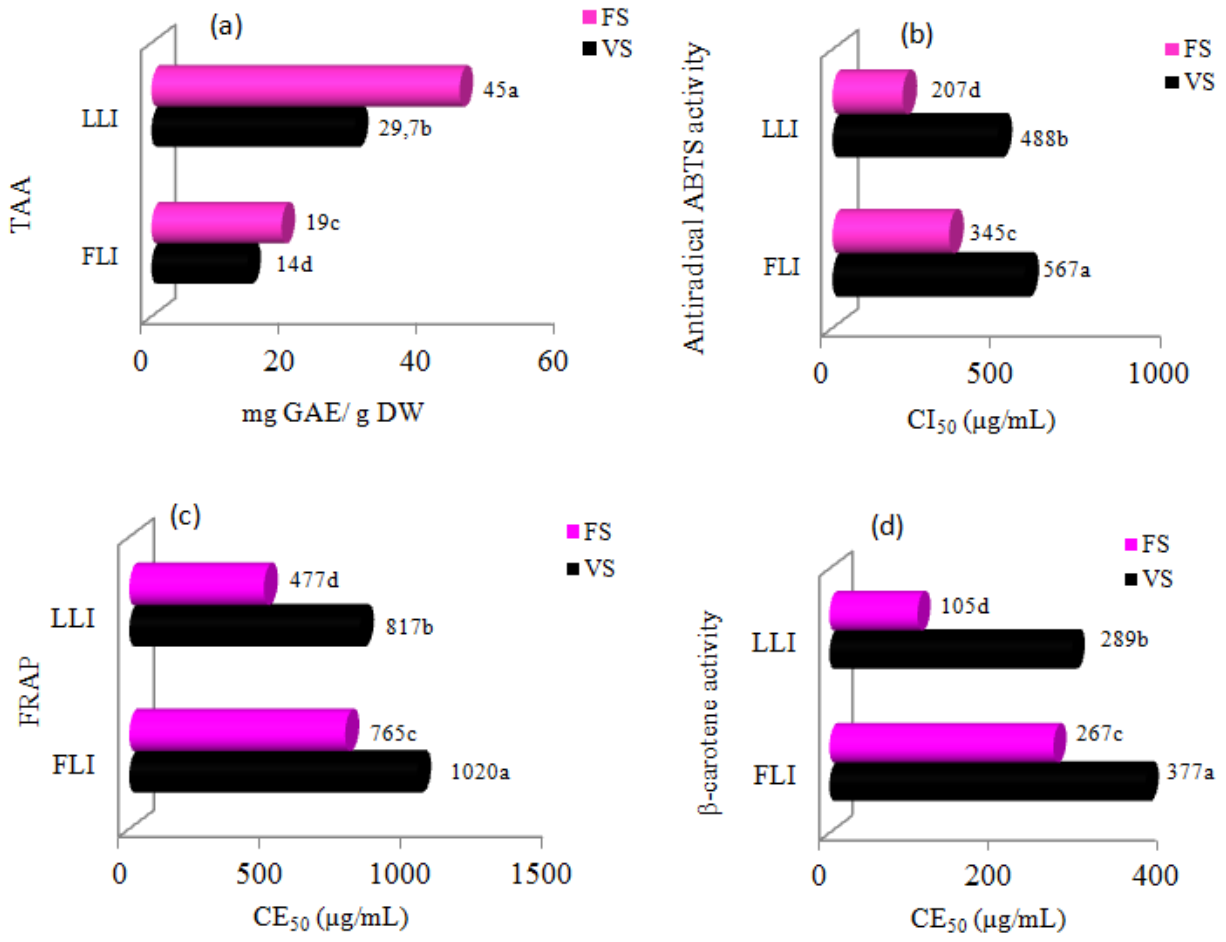

Figure 3. Changes in (a): Total antioxidant activity, (b): ABTS radical scavenging activities, (c): ferric reducing antioxidant power and (d): β-Carotene bleaching test cultivated under LLI conditions.

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; IC_{50}, the concentration of the extract causing 50% inhibition; EC_{50}, the effective concentration at which the absorbance was 0.5; *Values are means of three replicates and standard deviation. Values with different superscripts (a-d) are significantly different at P < 0.05.*

Table 2. Changes in MDA, EL, total polyphenols, flavonoids and tannins in the leaves of C. tinctorius plants cultivated under LLI conditions. GAE, gallic acid equivalent; CE, catechin equivalent; MDA, malondialdehyde; TCT, total condensed tannin, TFC, total flavonoids; TPC, total polyphenol

	FLI		LLI	
	Vegetative	Flowering	Vegetative	Flowering
MDA (nmol/ gFW)	7.3±0.07^{c}	10.33±0.07^{c}	8.9±0.07^{c}	13.89±0.07^{c}
EL %	25±0.07^{c}	34±0.07^{c}	37±0.07^{c}	56±0.07^{c}
TPC (mg GAE mg^{-1} DW)	21.20±1.80^{c}	19.13±0.07^{c}	35.42±1.98^{b}	41.19±1.15^{a}
TFC (mg CE mg^{-1} DW)	7.81±0.55^{a}	10.72±1.14^{b}	13.65±0.02^{c}	23.74±0.00^{d}
TCT (mg CE mg^{-1} DW)	13.61±0.26^{a}	15.12±2.11^{b}	16.10±0.33^{c}	21.44±1.98^{d}

Values are means of three replicates and standard deviation. Values with different superscripts (a-d) are significantly different at $P < 0.05$.

DISCUSSION

Low light was shown to substantially distress the agronomic traits of plants and restrain physiological metabolic processes, including photosynthesis and antioxidant characteristics, as well as carbon and nitrogen fixation; it can influence plant growth and development by altering the plant niche and its most visual effect is plant morphological change (Wang et *al.*, 2012). Previous studies have suggested that shaded plants preferentially supplied photosynthetic products to leaves that were beneficial to their growth, which could partially compensate for the decrease in growth rate due to reduced light energy; additionally, shade could also decrease the plant canopy and distribute assimilated carbon to the vertical growth to furthest capture light energy (Zhu et *al.*, 2017). These observations were all confirmed by our present study when subjecting *C. tinctorius* to LLI resulted in a severe reduction of LDW in both growth stage phases (-94 and -80% respectively fo VS and FS).

In *Echinochloa colona* a reduction of -50% and -75% in LI led to a decrease of about 28% and 69% of the LDW. According to Zavala and Ravetta (2001), plants cultivated in shade supplied photosynthetic products to leaves in order to promote their growth; thus offsetting the reduction of plant growth induced by light deficiency.

Another factor that affects plant growth is the intensity of light that varies hugely depending on the season. Indeed, photosynthetic process depends deeply on the quantity of light received by plants which affects directly plant metabolism. In fact, photosynthetic activity generated essential organic compounds for plant growth, as described by Brüggemannet *al.* (2011). Thus, the reduction of plant growth under light deficiency could be explained by an insufficient production of ATP essential for carbon fixation and carbohydrate biosynthesis.

A decrease in ChL content with LLI has been detetcted in many species, such as the cape gooseberry (Aldana *et al.,* 2014), tomatoes black beans (Bansal *et al.,* 2019), and cabbage (Casierra-Posada and Cutler, 2017), which is manifested by foliar yellowing, followed by wilting, affecting photosynthesis (Wu *et al.,* 2015); thus reducing the biomass of plants, such as with a decrease in leaf area and root volume (Cardona *et al.,* 2016). LL stress causes different types and levels of damage to plant cells. One type involves leaf blade cells membrane destruction, which leads to increased cell permeability and intracellular conductivity. MDA, which is produced during lipid peroxidation, is an important index of cell damage under stress (Zhu et *al.,* 2017).

In the current study, MDA levels in safflower leaves was lower in shaded leaves at vegetative stage than in sun-exposed, which might be correlated with plant adaptive physiological regulation toshade which did not seem to harm *C. tinctorius* leaves at such stage. Although an increase in MDA contents at full flowering stage of low light-leaf development could help to dissipate excess excitation energy to a certain extent (Zhou et *al.,* 2004), it is also likely to result in an increase in excitation pressure of PS II and induce membrane-lipid peroxidation is related to the duration of

low light treatment and the degree of shaded light (Asada and Takahashi 1987), which is the peroxidation of unsaturated fatty acids in the membrane triggered by free radicals and thereby the production of peroxide toxic to cells, eventually might do some damage to photosynthetic apparatus. A remarkable increase of malondialdehyde (MDA), a product of membrane lipid peroxidation, was observed in cherry leaves when treated with just low light, indicating more serious peroxidation in the membrane (Huang et *al.,* 2002).

Phenolics play multiple chemical and biological functions in plants mainly related with adaptation to environmental changes since they represent a clear example of metabolic plasticity as plants are able to respond to external stresses rapidly inducing their biosynthesis in a reversible way (Wasli et *al.,* 2018). In this context, monitoring of total phenolic, flavonoïd and condensed tannin contents were assessed. As shown in the results, phenols, flavonoid and tannin levels were superior in shading leaves than to those exposed to full sunlight for both developmental stages. Such findings were in agreement with the resource allocation hypothesis proposed by Coley et *al.* (1985) that assumed that the production of flavonoids and phenolics would be up-regulated under low light conditions in *L. pumila.* It was suggested that increasing phenolic and flavonoid components in shading plants are related to lower temperatures under LL conditions. Chan et *al.* (2011) reported much greater concentrations of flavones and flavonols in leaves of vegetables that are exposed to shade. This finding is in agreement with Bergquist (2007), who showed that low irradiance use is important for the production of baby spinach with high in flavonoid concentration and composition.

On other hand, physiological stages can also affect polyphenols content and composition. We found that safflower at the flowering stage had a higher level of phenol than the vegetative stage. Similarly, Ichiho et *al.* (2013) accounted that phenol content of six crops cultivated in Japan was strongly affected by the growing season, Bano et *al.* (2013) also reported that secondary metabolites distribution may alter during plant development which may be related to the harsh climatic conditions of the plant's usual habitat which stimulate secondary metabolites biosynthesis. Phenolic content of a plant depends on a number of intrinsic (genetic, extracting solvent) and extrinsic(environmental, handling and development stage) factors (Medini et *al.,* 2013).

Hamrouni et *al.* (2009) concluded phenolic acids predominance during the early vegetative stage, whereas flavonoids predominated during other growth stages *O. majorana*. In another study, Ayan et *al.* (2007) reported that total phenol content reached the highest level at floral budding in *Hypericum hyssopifolium* and *Hypericum scabrum* and at full-flowering in *Hypericum pruinatum*. The findings of Verma and Kasera (2007) designated that peak concentration of phenols was observed in flowering stage in *Boerhavia diffusa* and *Sidacordifolia* except in *Asparagus racemosus* that showed maximal accumulation of phenols in the vegetative stage.

The antioxidant activities displayed a noteworthy difference from many studies. Indeed, Ben Mansour et *al.* (2018), found that leaves of *Cakile maritima* have an important antioxidant activity in the vegetative stage. This might suggest that these compounds play different roles depending on the state of the plant which could lead us to believe that, during flowering, these molecules are moving more toward a physiological role. While during the vegetative stage, they act preferentially as protector of plants by acting primarily as antioxidants.

CONCLUSION

No-enzymatic antioxidants and biological activities depend on several factors, mainly environmental conditions, and their light-tolerance are influenced by endogenous (ie, physiological development stage) and exogenous factors (i.e low light intensity). Our results demonstrated the influence of LL conditions and period of growth on dry weight and nutrient uptake content, evolution of phenolic pools and antioxidant activities of *C. tinctorius*. The stimulation of phenol amounts and antioxidant capacities confirmed the stimulation of their synthesis in absence of light and their role as protectors of plant structures against the oxidative stress. Besides floral stage proved an important activity in real food systems relative to commercially used, antioxidant extracts and economic feasibility of practical applications due to high phenolic contents. Therefore, the favored

harvest of safflower could be in F.S., which supports the utilization of this plant in a large field of application including cosmetic, pharmaceutical, agro alimentary and biological defense.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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