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Biomass production of lettuce: Nitrogen fertiliser and harvesting period effects on phytochemical composition in growth chamber-grown

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Abstract: Concern over the potential health benefits of the phytochemical compounds in lettuce has grown as intake of leafy vegetables, especially lettuce, has increased. This study was to investigate the effect of periods harvesting and the different dose of nitrogen fertilisation, including low, normal and high (100, 200 and 300 mg/L) respectively, on level of phytochemical compounds such as phenolic acids, flavonoids, carotenoids and chlorophylls in lettuce. The experiment was conducted in the growth chamber at Newcastle University. The plants were randomly designed for 3 treatments with 15 replications. Plants were harvested in three different periods. The first harvest took place after 6 weeks from planting, and the second and third harvests were done after 7 and 8 weeks from planting, respectively. After each harvest, the chlorophyll content and total carotenoids and other phytochemicals were measured by high performance liquid chromatography (HPLC) before and after drying the plants. The plant biomass was higher at normal nitrogen application (200 mg/L) in all periods of harvesting. As well as, significantly increased plant phytochemicals including carotenoids and chlorophylls, while, the composition of phenolic acids and flavonols were not affected such as, higher level of nitrogen fertiliser to the soil has resulted in a higher concentration of chlorophyll *a* and chlorophyll *b* in the lettuce by 154.2~420.3%, 72.1~287.9% respectively. As well as, that lutein was increased from 209 µg/g dry weight at the first period of harvest to 287 µg/g dry weight at the second period of harvest. Furthermore, the total phenolic increased by 25%. The findings of the current study showed that plant maturity has a positive correlation with plant phytochemicals.

Keyword: HPLC; *Lactuca sativa* L.; carotenoids; chlorophyll; phenolic acids

Lettuce (*Lactuca sativa* L.) is one of the most widely consumed green vegetables for salads. This is an annual plant belonging to the Asteraceae (Compositae) family. Lettuce's phytochemical profile has suitable amounts of several important components, including phenolic compounds, carotenoids, chlorophylls, vitamin A, C, and E, fibre, and minerals (Caldwell 2003; Oh et al. 2011). A number of epidemiological studies have shown that consumption of fruit and vegetables are as-

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sociated with lower occurrences of many common diseases, and these effects have been linked with the beneficial function of some phytochemicals which exist in fruit and vegetables (Coria-Cayupán et al. 2009).

However, there are many factors which affect the level of these phytochemicals in the plant. Numerous studies have reported that the accumulation of phenolic components and pigments (carotenoids and chlorophylls) in lettuce can be influenced by genetic/variety, agronomic factors including fertiliser, harvest, transplanting and so forth, and environmental factors including light, temperature, irrigation, soil management, humidity and so on (Zhao et al. 2007; Ribas-Agustí et al. 2011; Bumgarner et al. 2012). The influence of fertiliser application on the phytochemical compounds in horticultural crops, particularly lettuce, is not extensively studied and not yet completely understood. Most of them have reported that nitrogen fertiliser in both types (organic and inorganic) can increase yield and quality of lettuce significantly (Chohura, Kołota 2009; Testani et al. 2020). Whilst a study on the iceberg and romaine lettuce has reported that with increasing yield and quality, high nitrogen uptake result in felling post-harvest quality of lettuce (Hoque et al. 2010). However, the relationship between soil fertilisers (especially nitrogen) and the level of health promoting phytochemicals have extensively examined in many types of plant with different parts. Furthermore, the increase of nitrogen level in the soil associated with increased pigment contents in the lettuce, but in some cases reduced the level of total phenolic (Coria-Cayupán et al. 2009). Similarly, an experiment on the effect of organic and conventional fertilisers within an open field and high tunnel environment on the phenolic compounds in lettuce. The experiment has shown that both types of fertiliser were not consistently affective on phenolic compounds in lettuce (Zhao et al. 2007). However, there was a significant interaction between fertiliser type and growing conditions. The experiment has revealed that in an open field environment, inorganic fertiliser can increase the level of total phenolic in lettuce. Moreover, Becker et al. (2015) have conducted a study to determine the effect of nitrogen application level on the chlorophyll contents in the lettuce. The study has reported that increasing the amount of nitrogen fertiliser in the soil leads to a significant increase in the concentration of chlorophyll *a* and *b*. On the other hand, another important factor that affects phytochemical content in plants is the period of harvesting. According to Oh (2008) the content of total phenolic in lettuce decreases during plant ageing. Also, (Viacava et al. 2014) identified that the lettuce total phenolic is declined as long as the plant ages under open field and high tunnel conditions, but they mentioned that there was an increase of phenolic compounds just before harvesting. While, Rodriguez-Amaya et al. (2008) have stated that carotenoids content in lettuce relatively increases with plant maturity.

Hence, the aim of this study is to investigate the effect of periods of harvesting (reflected plant maturity) and the different concentration of nitrogen fertilisation including low, normal and high (100, 200 and 300 mg/L) respectively on level of phytochemical compounds such as phenolic acids, flavonoids, carotenoids and chlorophylls in lettuce grown in growth chamber with controlling environmental factors, such as the temperature and light.

MATERIAL AND METHODS

Growth media and nutrient solution. Irish peat moss was used as a growth media for lettuce. The pH of peat moss was very low (4.8) so that it was combined with lime to obtain the appropriate pH. The ratio of 1 : 30 (lime to peat moss) provided to reach pH (6.7) for the growth media. The nutrient solution was prepared based on Hoagland's nutrient solution with changing nitrogen concentration for three levels as: 100, 200 and 300 mg/L for low, normal and high concentration, respectively. The source of macronutrients (N, P, K) were: ammonium nitrate (NH_4NO_3) and of phosphorous salt instead of ammonium phosphate $(NH_4)_2PO_4$) and calcium nitrate ($Ca(NO_3)_2.4H_2O$), respectively (Jiménez-Pena et al. 2013)

Plant cultivation. Lettuce oak leaf 'Sansula' (*Lactuca sativa* L.) was sown in a growth chamber (Plant Growth/Environmental Chambers – MLR-351, SANYO, Japan). All plants (45 plants) were divided into 5 shelves in the growth chamber, and designed for 3 treatments: low, normal and high concentration of nutrient solution and 15 replications. Pots were filled with peat moss, and three seeds were added to each pot for germination. Seeds were covered by a thin layer of peat moss

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and irrigated carefully to keep moisture (within double concentration). The growth chamber was set for germination at a temperature of 20 °C for three days. As expected, germination occurred after three days. Then, four days after germination, unwanted plants were removed, keeping one plant per pot. The growth chamber was then set for plant growth at 22 °C, with a light intensity of 150 μmol/m^2/s. The photoperiod was 12/12 h day/night, and the temperature was 22 °C and 18 °C, day/night, respectively. The main source of light in the growth chamber was the vertical side (15 fluorescent light tubes). During plant growth, the light and humidity were measured. The light was measured by a photometer (Sunfleck, PAR Ceptometer, Model SF-80, Decagon Devices, Pullman, US) for different shelves. The average light intensity during the light period was 150 μmol/m^2/s. Due to different light conditions between different shelves and plant locations, the location of shelves and plants was changed every 10 days. The humidity was kept at 63.7% and recorded by a humidity meter (Ebro-a xylem brand, LEBI-20TH, Klipspringer, UK). Plants were irrigated by providing 50 mL of nutrient solution every two days from the day of planting until one day before harvesting (Qadir 2017)

Biomass determination. To determine fresh lettuce biomass, the samples were directly weighed after harvest and then transferred into a freezer (–80 °C) and kept until all the harvests were finished. Three days after the final harvest, all samples were transferred into a freeze-drier and kept for a week. The samples were dried at –20 °C until the pressure reached ≤ 0.06 mbar. After that, the samples were left at room temperature for 24 h, and the dried samples were weighed to determine the dry weight. The samples were ground by a coffee grinder (Wahl James Martin Spice Grinder, Wahl, United Kingdom).

Harvesting time. Plants were harvested in three different periods of growth. The first harvesting took place after 6 weeks from planting, and then the second and third harvesting were done after 7 and 8 weeks from planting, respectively. Each time, five plants of each treatment were picked randomly and then harvested.

HPLC analysis of chlorophylls and carotenoids content. The carotenoids and chlorophylls analysis in lettuce samples was done on the high-performance liquid chromatography (HPLC). The column was a Hyper Clone Reverse phase C18 (250 × 4.6 mm, 5 μm). The HPLC oven set at 40 °C with the flow at 1mL/min. The volume of injection was 20 μL, and the detection was at the wavelength of 450 nm. The solvents of the mobile phase included pure water (A), methanol (B), and ethyl acetate (C) (Park, Park 1997).

HPLC analysis of phenolic acids. The HPLC's column was a HyperClone Reverse phase C18 (250 × 4.6 mm, 5 μm), and the column oven was set at 25 °C. The photodiode array detector (PDA) was settled to collect all data from 200 to 600 nm. For each compound, the detection was controlled at 280 and 320 nm with a flow rate 0.1 mL/min. The injection volume for each sample was 20 μL into an HPLC, system equipped with a Shimadzu 2 LC-10AD pump (Shimadzu Corporation, Kyoto, Japan), SiL10A system Auto sampler (controller) (SiL10A, Shimadzu Corporation, Japan), a photodiode array UV-VIS detector (SPD-M10A, Shimadzu Corporation, Japan), a column oven (CTO-10AD, Shimadzu Corporation, Japan), and CLASS-VP chromatography software (Shimadzu Corporation, Japan). The mobile phase was 0.1% v/v trifluoroacetic acid (TFA)in ultrapure water (solvent A), 0.1% v/v trifluoroacetic acid in HPLC-grade acetonitrile (solvent B). The solvent gradient was: (H_2O + 0.1% TFA) (acetonitrile + 0.1% TFA), 0 min (100 : 0), 5 min (100 : 0), 15 min (83 : 17), 17 min (83 : 17), 22 min (75 : 25), 30 min (65 : 35), 35 min (50 : 50), 40 min (0 : 100), 50 min (0 : 100), 55 min (100 : 0) and 65 min (100 : 0) (Park, Park 1997)

Statistical analysis. All data were statistically analysed by using Minitab 16, ANOVA – general linear model (GLM). The effect of two different factors: levels of nitrogen fertiliser and different harvest times were tested. The Tukey test was used for comparison and interaction between factors. The $P < 0.05$ was considered a significant difference, whilst the $P < 0.01$ and $P < 0.001$ were considered a highly significant difference. All results were implemented by using three replicates for each level of fertiliser and harvest period. The tables show the mean ± standard deviation and the mean ± standard error. All the results were expressed as milligram per gram (dry and fresh weight).

RESULTS

Plant biomass

The obtained results showed that there were significant effects ($P < 0.05$) of levels of nitrogen

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fertiliser on the fresh and dry weight of lettuce. There were also highly significant effects ($P < 0.001$) of harvest period on the plant biomass, but a significant interaction between fertiliser and harvest periods was not observed, see Table 1.

Chlorophylls and carotenoids content

Chlorophylls. Nitrogen fertiliser had a significant effect on the concentration of chlorophyll *a*, chlorophyll *b* and total chlorophylls in lettuce. Although there was no significant effect of harvest period on the chlorophyll contents, and there was no interaction between nitrogen fertiliser and the period of harvesting. The effect was similar in dry and fresh weight lettuce, as shown in Table 2.

Carotenoids. In dry weight, there were significant effects of nitrogen fertiliser on the carotenoids content ($P < 0.05$), but the periods of harvesting had no significant effect, and there were no interactions between the nitrogen fertiliser and the periods of harvesting. On the other hand, in fresh weight, there were also significant effects of nitrogen fertiliser on the concentration of carotenoids, ($P < 0.05$), but the effect of periods of harvesting were significant ($P < 0.05$) only for lutein, cis beta-carotene and total carotenoids contents, whilst there was no interaction between factors, as shown in Tables 2.

Phenolic compounds (HPLC analysis)

Phenolic acids. The analysis showed that in dry weight, there were no significant effects of nitrogen concentration on the content of phenolic acids, while periods of harvesting on the phenolic acids accumulation, such as caftaric acid, chichoric acid and the sum of phenolic acids, were significant. Additionally, the interaction was significant between nitrogen concentration and periods of harvesting. Although in fresh weight, there were no significant effects of nitrogen concentration and periods of harvesting on phenolic acids, and there was no significant interaction between factors (Table 3).

Flavonoids. Analyses of flavonoids in dry weight showed there were just significant effects of periods of harvesting on the content of rutin ($P < 0.05$), and there was no significant effect of nitrogen fertiliser on the rutin and sum quercetins and no significant interaction was noted between factors. On the other hand, in fresh weight, there was neither a significant effect nor a significant interaction between both factors on the composition of flavonoids in lettuce (Table 3).

Table 1. Analysis of variance (ANOVA) for dry and fresh weight of lettuce grown with different levels of nitrogen fertiliser and harvested at different periods

Nitrogen concentration in nutrient solution (mg/L)	Harvest period	Plant biomass		
		fresh weight	dry weight	dry matter (%)
		(g/plant)		
100	first	4.760 ± 3.281	0.337 ± 0.214	7.08
200	first	5.226 ± 1.607	0.396 ± 0.086	7.57
300	first	4.028 ± 1.143	0.327 ± 0.087	8.12
100	second	17.242 ± 0.652	1.158 ± 0.115	6.72
200	second	18.060 ± 7.136	1.287 ± 0.530	7.13
300	second	13.377 ± 0.313	1.032 ± 0.043	7.74
100	third	30.318 ± 9.308	1.764 ± 0.515	5.82
200	third	34.439 ± 4.497	2.407 ± 0.356	6.99
300	third	23.005 ± 1.937	1.642 ± 0.184	7.14
ANOVA *P*-value				
Fertiliser		0.037	**0.044**	0.064
Harvest period		0.000	**0.000**	**0.012**
Fertiliser × harvest		0.401	0.288	0.787

The values are mean ± standard deviation within the same factor, the effect of levels of nitrogen fertilisers in nutrient solution (100, 200 and 300 mg/L) is significant ($P < 0.05$), and the effect of harvest periods are highly significant ($P < 0.001$); bold – the highest mean values recorded for each plant biomass parameter

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Table 2. Analysis of variance (ANOVA) for chlorophylls and carotenoids concentration in dry and fresh lettuce measured by HPLC and grown with different fertiliser levels and harvested at different periods

Nitrogen concentration in nutrient solution (mg/L)	Harvest period	Total chlorophylls and carotenoids measured by HPLC						
		lutein	beta-carotene	cis beta-carotene	chlorophyll *a*	chlorophyll *b*	total chlorophylls	total carotenoids
Dry lettuce (mg/g dry weight)								
100	first	0.346 ± 0.049	0.112 ± 0.018	0.049 ± 0.009	14.520 ± 1.205	2.622 ± 0.187	17.142 ± 1.392	0.507 ± 0.075
200	first	0.301 ± 0.052	0.092 ± 0.007	0.040 ± 0.004	13.118 ± 2.518	2.532 ± 0.767	15.650 ± 3.281	0.433 ± 0.060
300	first	0.358 ± 0.088	0.104 ± 0.021	0.049 ± 0.008	15.796 ± 3.058	3.036 ± 0.669	18.831 ± 3.726	0.512 ± 0.118
100	second	0.296 ± 0.029	0.096 ± 0.012	0.039 ± 0.004	13.893 ± 1.190	2.459 ± 0.186	16.352 ± 1.367	0.431 ± 0.044
200	second	0.293 ± 0.037	0.091 ± 0.018	0.037 ± 0.007	14.342 ± 2.007	2.590 ± 0.290	16.932 ± 2.295	0.423 ± 0.061
300	second	0.350 ± 0.043	0.116 ± 0.017	0.047 ± 0.005	16.930 ± 2.141	2.944 ± 0.340	19.874 ± 2.480	0.512 ± 0.065
100	third	0.278 ± 0.008	0.087 ± 0.010	0.039 ± 0.002	14.322 ± 0.733	2.581 ± 0.188	16.903 ± 0.890	0.405 ± 0.008
200	third	0.323 ± 0.048	0.100 ± 0.024	0.044 ± 0.009	16.241 ± 2.027	2.905 ± 0.391	19.146 ± 2.406	0.467 ± 0.081
300	third	0.374 ± 0.051	0.118 ± 0.018	0.050 ± 0.008	18.474 ± 2.347	3.358 ± 0.466	21.832 ± 2.771	0.542 ± 0.075
ANOVA *P*-value								
Fertiliser		**0.046**	0.081	**0.035**	**0.017**	**0.034**	**0.018**	**0.048**
Harvest period		0.639	0.974	0.261	0.166	0.372	0.199	0.690
Fertiliser × harvest		0.548	0.340	0.452	0.671	0.921	0.725	0.478
Fresh lettuce (mg/g fresh weight)								
100	first	0.026 ± 0.005	0.009 ± 0.002	0.004 ± 0.001	1.113 ± 0.190	0.201 ± 0.034	1.315 ± 0.224	0.039 ± 0.007
200	first	0.023 ± 0.001	0.007 ± 0.002	0.003 ± 0.001	0.994 ± 0.050	0.189 ± 0.027	1.183 ± 0.068	0.033 ± 0.002
300	first	0.029 ± 0.004	0.008 ± 0.001	0.004 ± 0.001	1.273 ± 0.096	0.244 ± 0.026	1.517 ± 0.122	0.041 ± 0.005
100	second	0.020 ± 0.003	0.007 ± 0.001	0.003 ± 0.001	0.931 ± 0.097	0.165 ± 0.013	1.096 ± 0.109	0.029 ± 0.004
200	second	0.021 ± 0.002	0.006 ± 0.002	0.003 ± 0.001	1.015 ± 0.106	0.184 ± 0.014	1.199 ± 0.120	0.030 ± 0.003
300	second	0.027 ± 0.004	0.009 ± 0.002	0.004 ± 0.001	1.306 ± 0.174	0.227 ± 0.028	1.533 ± 0.203	0.040 ± 0.005
100	third	0.016 ± 0.001	0.005 ± 0.001	0.002 ± 0.001	0.836 ± 0.029	0.151 ± 0.007	0.987 ± 0.033	0.024 ± 0.001
200	third	0.023 ± 0.004	0.007 ± 0.002	0.003 ± 0.001	1.136 ± 0.169	0.203 ± 0.032	1.339 ± 0.201	0.033 ± 0.007
300	third	0.027 ± 0.004	0.008 ± 0.002	0.003 ± 0.001	1.317 ± 0.182	0.239 ± 0.032	1.556 ± 0.212	0.038 ± 0.006
ANOVA *P*-value								
Fertiliser		**0.001**	**0.045**	**0.012**	**0.000**	**0.000**	**0.000**	**0.001**
Harvest period		**0.031**	0.189	**0.012**	0.789	0.267	0.691	**0.026**
Fertiliser × harvest		0.100	0.255	0.315	0.128	0.318	0.143	0.101

HPLC – high performance liquid chromatography; the values are mean ± standard deviation within same factor, the effect of levels of nitrogen fertiliser (low: 100, normal: 200 and high: 300 mg/L) is significant ($P < 0.05$), but there is no significant effect of periods of harvesting on the content of chlorophylls and carotenoids in fresh lettuce; there is no significant interaction ($P > 0.05$) between nitrogen fertiliser and harvest periods; bold – the highest mean concentration recorded for each chlorophyll and carotenoid compound

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Table 3. Analysis of variance (ANOVA) for phenolic acids and flavonoids concentration in dry and freah lettuce measured by HPLC, and grown with different fertiliser levels and harvested at different periods

Nitrogen concentration in nutrient solution (mg/L)	Harvest period	Phenolic acids and flavonoids measured by HPLC					
		caftaric acid	chlorogenic acid	chichoric acid	rutin	sum phenolic acids	sum quercetin
Dry lettuce (mg/g dry weight)							
100	first	0.859 ± 0.104	0.268 ± 0.081	2.406 ± 0.106	0.322 ± 0.075	4.147 ± 0.070	0.549 ± 0.147
200	first	0.936 ± 0.419	0.263 ± 0.155	3.277 ± 2.405	0.345 ± 0.178	5.111 ± 3.342	0.596 ± 0.336
300	first	0.464 ± 0.097	0.403 ± 0.484	1.337 ± 0.526	0.210 ± 0.024	2.509 ± 1.010	0.403 ± 0.016
100	second	1.185 ± 0.409	0.436 ± 0.213	4.287 ± 2.484	0.424 ± 0.225	6.554 ± 3.313	0.694 ± 0.341
200	second	0.997 ± 0.214	0.422 ± 0.079	3.869 ± 1.744	0.453 ± 0.142	5.827 ± 2.096	0.694 ± 0.172
300	second	1.072 ± 0.059	0.747 ± 0.269	5.465 ± 0.869	0.543 ± 0.131	7.918 ± 1.184	0.785 ± 0.124
100	third	1.184 ± 0.492	0.282 ± 0.108	4.023 ± 2.477	0.432 ± 0.122	6.126 ± 3.344	0.761 ± 0.209
200	third	1.161 ± 0.257	0.449 ± 0.220	5.004 ± 2.148	0.483 ± 0.152	7.243 ± 2.762	0.765 ± 0.204
300	third	1.035 ± 0.048	0.534 ± 0.264	4.256 ± 1.824	0.433 ± 0.135	6.422 ± 2.272	0.664 ± 0.187
ANOVA *P*-value							
Fertiliser		0.250	0.124	0.847	0.850	0.903	0.788
Harvest period		**0.023**	0.170	**0.033**	**0.030**	**0.039**	0.081
Fertiliser × harvest		0.579	0.874	0.562	0.630	0.563	0.804
Fresh lettuce (mg/g fresh weight)							
100	first	0.065 ± 0.007	0.021 ± 0.010	0.184 ± 0.027	0.024 ± 0.003	0.319 ± 0.056	0.041 ± 0.006
200	first	0.076 ± 0.046	0.022 ± 0.017	0.276 ± 0.246	0.028 ± 0.020	0.426 ± 0.348	0.049 ± 0.037
300	first	0.039 ± 0.013	0.031 ± 0.034	0.108 ± 0.035	0.017 ± 0.001	0.202 ± 0.067	0.033 ± 0.005
100	second	0.080 ± 0.031	0.030 ± 0.016	0.289 ± 0.178	0.029 ± 0.017	0.443 ± 0.239	0.047 ± 0.025
200	second	0.071 ± 0.012	0.030 ± 0.005	0.272 ± 0.111	0.032 ± 0.009	0.410 ± 0.131	0.049 ± 0.010
300	second	0.083 ± 0.003	0.057 ± 0.019	0.420 ± 0.056	0.042 ± 0.009	0.609 ± 0.073	0.060 ± 0.008
100	third	0.070 ± 0.031	0.016 ± 0.007	0.237 ± 0.152	0.025 ± 0.008	0.360 ± 0.205	0.045 ± 0.013
200	third	0.081 ± 0.017	0.031 ± 0.015	0.348 ± 0.147	0.034 ± 0.010	0.504 ± 0.188	0.053 ± 0.014
300	third	0.074 ± 0.006	0.038 ± 0.020	0.305 ± 0.137	0.031 ± 0.010	0.460 ± 0.172	0.048 ± 0.014
ANOVA *P*-value							
Fertiliser		0.614	0.077	0.638	0.591	0.706	0.738
Harvest period		0.236	0.242	0.113	0.136	0.161	0.417
Fertiliser × harvest		0.424	0.762	0.396	0.473	0.396	0.731

HPLC – high performance liquid chromatography; the values are mean ± standard deviation within same factor, the effect of levels of nitrogen fertiliser (low: 100, normal: 200 and high: 300 mg/L) are no significant; nevertheless, the effects of period of harvesting are significant ($P < 0.05$) except for chlorogenic acid and sum quercetins in dry weight lettuce; there is no significant interaction between nitrogen fertiliser and harvest periods; bold – highest mean concentration recorded for each phenolic acid and flavonoid compound

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DISCUSSION

Plant biomass

The effects of the experiment in the present study showed that nitrogen concentration and periods of harvesting significantly influenced plant biomass. The plant biomass was higher at a normal nitrogen dose (200 mg/L) in all periods of harvesting. Regarding the dry weight, the highest dry weight was obtained from the third period of harvesting (8 weeks after sowing), 2.407 g dry weight/plant, whilst the lowest was obtained at the first period of harvesting, 0.327 g dry weight/plant. These results agree with the previous studies. In a study (Mitchell et al. 1991), nitrogen enhanced leaf dry weight and increased by 32% in comparison with the control. Additionally, in another investigation, the effect of the nitrogen fertilizer and period of harvesting on the plant biomass including 14, 28, and 41 days after snowing, they mentioned that period of harvesting with or without nitrogen fertiliser increased plant biomass 50.5, 424.9 and 649.8 g/m^2 fresh shoot weight of lettuce from the first harvesting to the final harvesting, respectively (Bumgarner et al. 2012).

Furthermore, in fresh-weight lettuce, the effects were similar. Generally, in the current study, it has been observed that a normal concentration of nitrogen application (200 mg/L) contributed to an increment in the plant biomass, whereas reduced plant biomass was observed at high nitrogen application, 300 mg/L (Konstantopoulou et al. 2010). It was concluded that 200 mg/L of nitrogen in the nutrient solution for lettuce growth is optimal due to increasing yield and quality of lettuce.

Phytochemicals

Chlorophylls. Kleinhenz et al. (2003) have reported that chlorophyll content lessens with plant age. This disagreement might be due to the effect of light density on the plant in the present study, because after each harvesting, the distance between plants was wider, and that was in favour of the plants receiving more light intensity than previously harvested plants. Caldwell and Britz (2006) have confirmed that supplementation of ultraviolet radiation in the light spectrum causes an increment in the content of chlorophylls in the lettuce.

Carotenoids. In the current study, the increase of carotenoid content was observed, and there is strong evidence in addition to previous studies about the relationship between nitrogen fertiliser and carotenoids composition. A study on the effect of nitrogen application on secondary plant metabolites in lettuce demonstrated that nitrogen treatments resulted in higher carotenoid contents than control (Coria-Cayupán et al. 2009). Furthermore, low nitrogen application is usually associated with decreasing the total carotene contents in lettuce (Gross 2012).

Nonetheless, periods of harvesting have a significant effect on the lutein, cis beta-carotene and total carotenoids concentration. In dry weight, the carotenoids, which were measured by HPLC, were decreased at the second harvest period and increased at the third harvest time, particularly lutein and beta-carotene. In comparison with previous studies such as (Nicolle et al. 2004) who have reported that carotenoid composition was affected by the period of harvesting. They reported that lutein content in butter lettuce was increased from 209 μg/g dry weight at the first period of harvest to 287 μg/g dry weight at the second period of harvest.

Phenolic compounds

In the present study, chichoric acid was observed as the main phenolic acid in the lettuce. There was no significant effect of nitrogen level in the nutrient solution on the content of phenolic acids and flavonols. This observation disagrees with the previous studies done on vegetables and fruit. According to the hypothesis of carbon-nitrogen balance, which supports that change in the balance between carbon and nitrogen results in a transition of plant ingredient from primary to secondary plant metabolite, therefore, reduction of plant growth stimulated by low nitrogen availability leads to an increase carbon in plants which 'are used for making carbon-based secondary metabolites like phenolic and terpenes' (Oh 2008). In accordance with the previous studies on lettuce, it can be mentioned that the effect of nitrogen fertiliser on the phenolic compounds is not consistent. nitrogen application in some cases reduces the level of phenolic compounds (Qadir et al. 2017).

However, the different periods of harvesting and thus the plant maturity led to an increase in phenolic composition analysed in fresh weight lettuce, but this effect was not statistically significant. Whilst in dry weight lettuce, periods of har-

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vesting significantly influenced this parameter. The differences between fresh weight and dry weight lettuce may be due to the effect of post-harvest storage on the concentration of phenolic compounds. As it was reported by (Viacava et al. 2018) 2 weeks after post-harvest storage total phenolics increased by 25%. Overall, in the present study, periods of harvesting resulted in an increase in phenolic composition in lettuce. These results concur with those reported by Koukounaras et al. 2016). They have reported that different harvest period had marginal effect on the total phenolic contents in green cultivars specially on caftaric acid concentration in dry weight.

CONCLUSION

In this factorial study, nitrogen fertiliser and plant age had a positive relation with carotenoids concentration, such as lutein, beta-carotene, cis beta-carotene and total carotenoids; however, it had no effect on beta-carotene. Furthermore, a similar effect was observed for the chlorophyll contents. In the subject of phenolic acids and flavonoids, unexpected results were observed, and nitrogen application had no significant effect on the composition of phenolic acids and flavonoids. The reason for this effect may refer to the inconsistent effect of fertiliser on the phenolic compounds in lettuce. On the other hand, the concentration of other phytochemicals compounds in lettuce such as phenolic acids and flavonols were partly affected by plant maturity. According to these results, it can be mentioned that increasing the nitrogen concentration in the nutrient solution and plant age enhances the content the health promoting phytochemicals, which can be favourable in the human diet.

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