KIRKUK UNIVERSITY JOURNAL FOR AGRICULTURAL SCIENCES

Impact of Urea Supplements and Cultivars on Yield, Nitrate Accumulation and Some Phytochemical Properties in Lettuce Grown under Greenhouse Conditions

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- Date of research received 31 /03/2023 and accepted 24/04/2023.

Abstract

A greenhouse experiment was assayed to study the impact of urea supplements 100, 200, and 300 kg ha^{-1} and cultivars Nadir, Polaris, and Fajir on the yield, nitrate accumulation and some phytochemical contents in lettuce. The results indicated that all supplemental urea treatments significantly improved stem diameter, chlorophyll intensity in outer leaves, plant yield and total marketable yield. In addition, the urea doses markedly increased nitrate accumulation in leaves, while its accumulation declined at the highest level compared to the other doses. Furthermore, the TPC significantly decreased at the high dose of urea supplement. Additionally, the urea supplement, especially 100 and 300 kg ha^{-1}, notably increased TFC. The Polaris cultivar showed the highest stem diameter, chlorophyll intensity in the inner leaves, dry matter percentage, plant yield, total marketable yield as well as accumulated minimal nitrate in their leaves. Moreover, the highest content of TPC and TFC was obtained by Nadir cultivar. In addition, the Fajir cultivar recorded the highest activity of total antioxidants. Finally, the interaction between Polaris cultivar and 200 kg ha^{-1} urea maintained a good balance between the yield and its quality. As a result, the findings of this study may help our growers adopt the optimum urea concentrations with lettuce cultivars in their fields under greenhouse circumstances.

Keywords: *Lactuca sativa* L., Productivity, NO_3, Antioxidants, TPC, TFC, DPPH

Citation: Halshoy, H., Sulaiman, S., Haidar Arkwazee, H., & Mahmood, A. (2023). Impact of Urea Supplements and Cultivars on Yield, Nitrate Accumulation and Some Phytochemical Properties in Lettuce Grown under Greenhouse Conditions. *Kirkuk University Journal For Agricultural Sciences*,14(2), 35-53. doi: 10.58928/ku23.14204.
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Introduction

Lettuce (*Lactuca sativa* L.) is a leafy annual plant belongs to the *Asteraceae* family. It is one of the most popular vegetables in terms of consumption rate and economic importance worldwide [1]. At the end of 2019, the average production of lettuce in Iraq is about 7.05 ton ha^{-1} and the total production was 31232 tons which is considered low productivity compared with neighboring countries [2]. Lettuce contains few calories, fat, and sodium, as well as it is high in fiber, iron, folate, and vitamin C. It is also a good source of a variety of other health-promoting bioactive compounds such as phenols and flavonoids. The bioactive compounds in lettuce have anti-inflammatory, anti-diabetic, and cholesterol-lowering properties [3] and [4]. However, numerous studies have indicated that the accumulation of these healthy compounds is strongly influenced by the growing conditions, cultivars, and nutrient availability in the soil [5].

Nitrogen (N) is an essential nutrient for plant growth and development. It is also the most abundant nutrient in plants which constitutes 2-4% of plant dry matter [6]. It is found in many biomolecules such as amino acids, proteins, nucleic acids, chlorophyll, ATP, and phytohormones. A large part of the plant body is composed of N-containing compounds [7]; which are necessary to complete the biological processes involving carbon and nitrogen metabolisms, photosynthesis, and protein production [8]. Generally, vegetable crops require adequate amounts of N; in addition, the extra availability of N which is not tailored to the plant requirement may reduce product quality through nitrate accumulation in the edible parts [9] and [10].

Leafy vegetables, including lettuce, are considered to accumulate higher nitrate than other vegetables, which may harmful to human health while converting to nitrite and nitric oxide by salivary enzymes and oral bacteria [11]. According to the European Union Food Commission, the Acceptable Daily Intake (ADI) level of nitrate is about 3.70 mg kg^{-1} of body weight [12]. Additionally, the U.S. Environmental Protection Agency (EPA) reported that the acceptable daily consumption of nitrate is roughly 7.0 mg kg^{-1} of body weight [11] and [13].

Furthermore, the phytochemical contents such as the phenol and flavonoid contents as well as antioxidant activity in lettuce leaves are highly affected by N supplementation. Studies found that the phenol and flavonoid contents increased by reducing N concentration [14] and [15]. In this regard, the aim of this study is to evaluate the effect of urea supplements as a source of N on the yield, nitrate accumulation and some phytochemical characteristics of three commercial lettuce cultivars under greenhouse conditions in Sulaimani governorate.

Materials and Methods

Experimental Site and Soil Analysis

This experiment was laid out during the period of November 5th, 2021to March 3rd, 2022 at the research farm belongs to Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region, Iraq. The study was conducted in a plastic-house (330 m^2) covered with 200μm polyethylene plastic film. Soil samples from the study area were collected at a depth of (0-30 cm, 14 sub samples) to determine some physical and chemical properties. Prior to analysis, samples were air dried and passed through a 2 mm sieve. Results of the soil analysis are shown in Table 1.

Table 1. Some physical and chemical characteristics of the experiment soil

Properties	Sand	Silt	Clay	Texture	pH	EC	Organic Matter	$CaCO_3$	Total N	Available P	Soluble K
Unites	%	%	%	---	---	dS m^{-1}	g kg^1	g kg^1	g kg^1	mg kg^1	g kg^1
Values	9.8	43.9	46.3	Silty-Clay	7.9	1.04	10.9	267	13.7	5.6	56.4

Soil Preparation, Transplanting and Treatments Detail

After plowing, smoothing and leveling the soil of the greenhouse; the soil was divided into three terraces where each of them considered as a replicate. After that, the drip irrigation system was installed and the terraces were mulched with black polyethylene plastic film. Each replicate was separated to 12 experimental units and a distance of 120 cm was left between them. After the experiment setup, three cultivars of lettuce Nadir, Polaris, and Fajir were transplanted on November 5th, 2021. In addition, each experimental unit contained six plants that were cultivated in two lines and space between the plants within a line was 40 cm. Furthermore, each cultivar in each replicate was fertilized with three levels of urea fertilizer (100, 200 and 300 kg ha^{-1}) by soil-drenching method in addition to the control treatment. The first application was implemented after 14 days of transplanting, and the others with 21 days intervals.

Measurements

At the end of the growing season, three plants in each plot were selected randomly and the following characteristics were measured: stem diameter, number of unfolded leaves per plant, chlorophyll intensity of outer and inner leaves, leaves dry matter percentage, total soluble solid (TSS), plant yield, total marketable yield, nitrate accumulation, total phenolic content (TPC), total flavonoid content (TFC) as antioxidants as well as total antioxidant activity (TAA) of the leaves.

The outer and inner leaves chlorophyll intensity was detected by using a digital chlorophyll meter (SPAD-502; Minolta, Osaka, Japan). Also, a digital refractometer (Model: PAL-1, Atago, Tokyo Tech., Japan) was used to measure the TSS. Furthermore, to determine leaf dry matter percentage the leaf samples were placed in the forced-air oven (Model: LOD-250N, LabTech®, Korea) at 65˚C till the weight became constant, the dried leaves were weighted using electronic balance then the percentage of the leaf dry matter were calculated based on the following equation:

$$\textbf{\textit{Leaf dry matter}}\ (\%) = \frac{\textbf{\textit{leaf dry weight}}}{\textbf{\textit{leaf fresh weight}}} \times \mathbf{100} \qquad (\mathbf{1})$$

Nitrate concentration in the leaves was assayed according to [16]. For determining TPC, TFC and TAA in the leaves, the method of [17] and [18] was used for preparing the sample extracts as follows: leaf samples were snap-frozen by using liquid nitrogen. Frozen samples were ground to fine powder using pestle and mortar. After that, 1 g of ground powders were taken and placed in 15 ml tubes, then 10 ml of 80% methanol was added into the tubes and shaken in a water bath for 3 hours at 38˚C. The mixture was centrifuged at 5000 rpm for 15 minutes at 4°C. The upper layer (extracts) was transferred into new tubes and they were kept in a refrigerator at 4°C as a crude extracted solution for antioxidants analysis.

TPC in each extract was determined according to the Folin-Ciocalteu method with some modifications [19]. Briefly, 100 µl of each extract was mixed with 4 ml of Folin-Cioculate reagent (Thomasbaker, India), and allowed to react for 5 minutes at room temperature. After that, 2 ml of 20% Na_2CO_3 solution was added then left for 50 minutes in the dark at 38 °C. Regarding the blank, the same previous steps were repeated except 100 µl of distilled water was used instead of the sample extracts. The absorbance of the reaction mixture was recorded at 765 nm by using spectrophotometer (Thermo Electron, UK). The standard solutions of gallic acid were prepared to create a standard curve and linear regression between the absorbance values at 765 nm and the gallic acid concentrations. The TPC was calculated and expressed as micrograms of gallic acid equivalent (GAE) per gram of dry weight of the leaf samples (µg GAE g^{-1} DW) by using the following equation:

$$\boldsymbol{TPC\ (\mu g\ GAE\ g-1\ DW) = C \times \frac{V}{W}} \ldots (\mathbf{2})$$

Where:

C: is the concentration of gallic acid determined from the standard curve,

V: is the volume of the extracts, and

W: is the dry weight of the samples

Furthermore, TFC was determined by the aluminum chloride ($AlCl_3$) colorimetric method [20] with slight modifications. An aliquot of 0.5 ml of each leaf sample extracts was added to the mixture of 1.5 ml methanol (80%), 0.1 ml of 10% (w/v) aluminum chloride ($AlCl_3$) solution, 0.1 ml of (1M) potassium acetate (CH_3COOK), and 2.8 ml of distilled water. The blank contained the same amount of mentioned chemicals with 0.5 ml of distilled water instead of the leaf extracts. The mixture was incubated at room temperature for 45 minutes. Thereafter, the absorbance of the reaction mixture was registered at 415 nm using a spectrophotometer (Thermo Electron, UK). The standard solutions of quercetin were prepared to achieve a standard curve and linear association between the absorbance values at 415 nm and the quercetin concentrations. The TFC was calculated and the results were expressed as micrograms of quercetin equivalent (QE) per gram of the dry leaf weight (µg QE g^{-1} DW) by using the following equation:

$$\boldsymbol{TFC\ (\mu g\ QE\ g-1\ DW) = C \times \frac{V}{W}}\ (\mathbf{3})$$

Where:

C: is the concentration of quercetin determined from the standard curve,

V: is the volume of the extracts, and

W: is the dry weight of the samples

The TAA of the leaf extracts was determined according to the DPPH (1-diphenyl-2-picrylhydrazyl) method which described by [21] 30µl of the leaf extracts was added to 1.7ml of methanolic solution of DPPH. The mixture was shaken vigorously and then incubated for 30 minutes at room temperature in the dark condition. The absorbance at 517 nm was registered. Total antioxidant activity was measured as inhibition percentage of the DPPH radical using the following equation:

$$Inhibition\ (\%) = \frac{ABS\ 517\ of\ control - ABS\ 517\ of\ sample}{ABS\ 517\ of\ control} \times 100 \quad (4)$$

Data Analysis

The experiment was conducted in a randomized complete block design (RCBD) with three replications, each replication consisted of 12 plots and the treatments were distributed randomly within each replicate. The collected data were submitted to the analysis of variance (Two-way ANOVA), and Duncan's new multiple range test at ($p < 0.05$) was used to compare the means. Furthermore, multiple correlation test and principal component analysis (PCA) were implemented using XLSTAT statistical analysis software (version 2019.2.2).

Results

Effect of urea supplements on the studied variables

The data in (Figure 1A) showed that urea supplement treatments significantly influenced on stem diameter of lettuce plants relative to control. This character significantly increased with increasing urea doses, in which lettuce plants treated with 300 kg ha^{-1} gave the highest stem diameter (44.03 mm) and compared to untreated plants increased by 13.2%. In addition, urea applications at all levels did not show significant differences in number of unfolded leaves per plant (Figure 1B). Furthermore, in comparison to untreated plants, the urea supplement doses increased chlorophyll intensity in the outer leaves by 21.2, 20.4 and 27.5% for 100, 200 and 300 kg ha^{-1} respectively (Figure 1C). While concerning the chlorophyll intensity in the inner leaves, the treatment of 300 kg ha^{-1} urea recorded the highest value (4.07 SPAD) which substantially different with the control and it was surpassed it by 28.4% (Figure 1D). Additionally, leaves dry matter percentage was not affected by urea supplements (Figure 1E). Nevertheless, the level of 200 kg ha^{-1} urea decreased TSS in the leaves by 16.8% (Figure 1F).

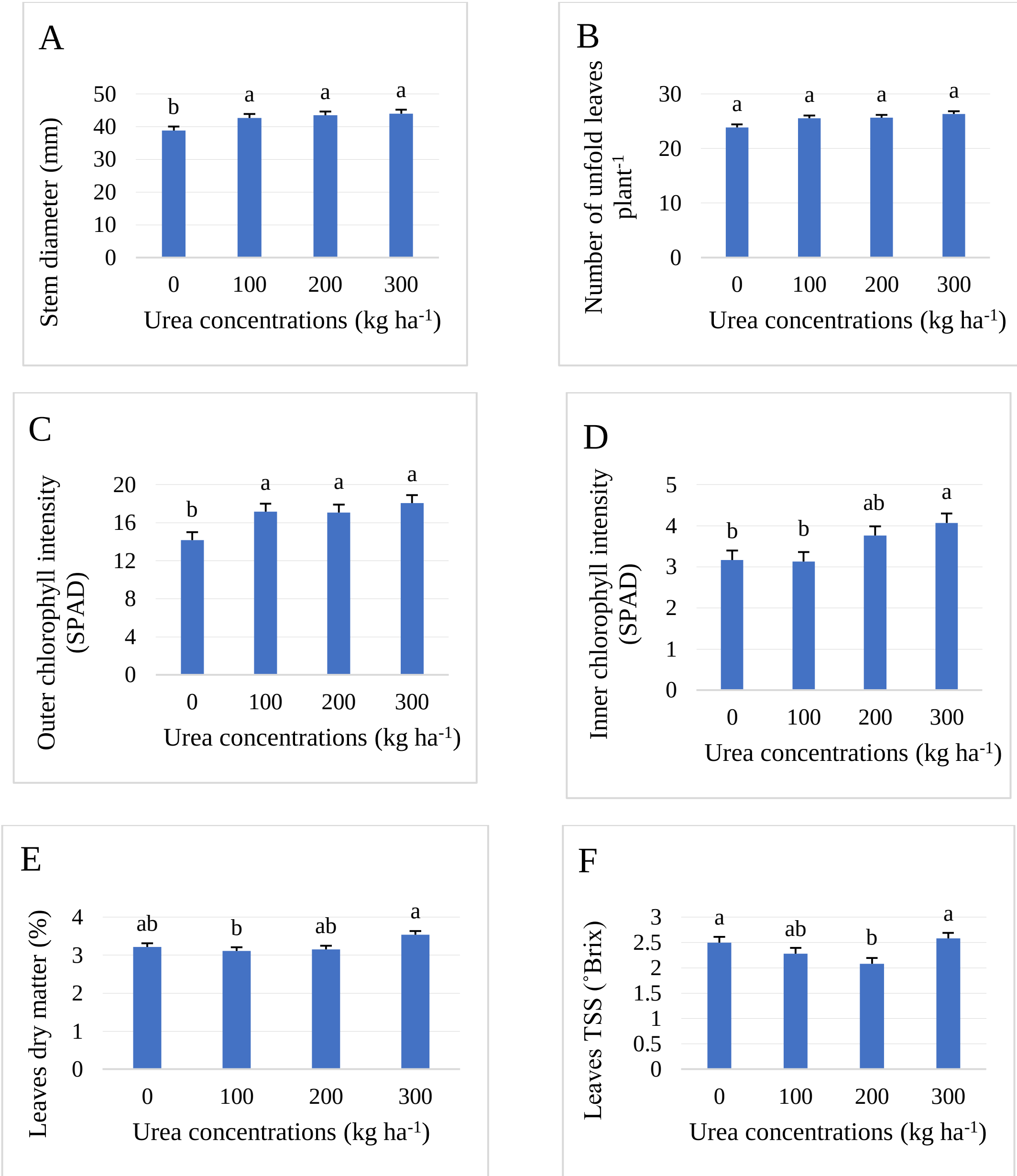


Figure 1. *Effect of urea concentrations on (A) stem diameter, (B) number of unfold leaves per plant, (C) outer leaves chlorophyll intensity (D) inner leaves chlorophyll intensity, (E) leaves dry matter, and (F) leaves TSS of the lettuce plant. Different letters between columns indicate significant differences at ($P < 0.05$).*

The findings in (Figure 2) show the influence of urea supplements on yield, nitrate accumulation and some phytochemical contents in the lettuce leaves. Results showed that the variations were not significant in yield and total marketable yield among the urea doses, while compared to control plants they increased yield as well as total marketable yield by 30.4, 46.3 and 32.7%, respectively for the dosages of 100, 200 and 300 kg ha^{-1} (Figure 2A and 2B). Furthermore, urea doses were affected markedly on increasing nitrate accumulation in the leaves. The levels of 100 and 200 kg ha^{-1} raised nitrate concentration by 46.3 and 82.1%, respectively compared to control plants. However, its accumulation declined at the highest level compared to other doses (Figure 2C). Concerning antioxidants content, our results indicated that the levels of 100 and 200 kg ha^{-1} urea were not different from untreated plants in TPC in the leaves, while the level of 300 kg ha^{-1} decreased it significantly by 13.6% compared to control (Figure 2D). In addition, the lettuce plants treated with 200 kg ha^{-1} urea was not different with control in TFC while the other levels; 100 and 300 kg ha^{-1}, were increased it by 11.3 and 14.3% respectively (Figure 2E). The urea supplements were not different from the control in terms of inhibition percentage of the DPPH radical (Figure 2F).

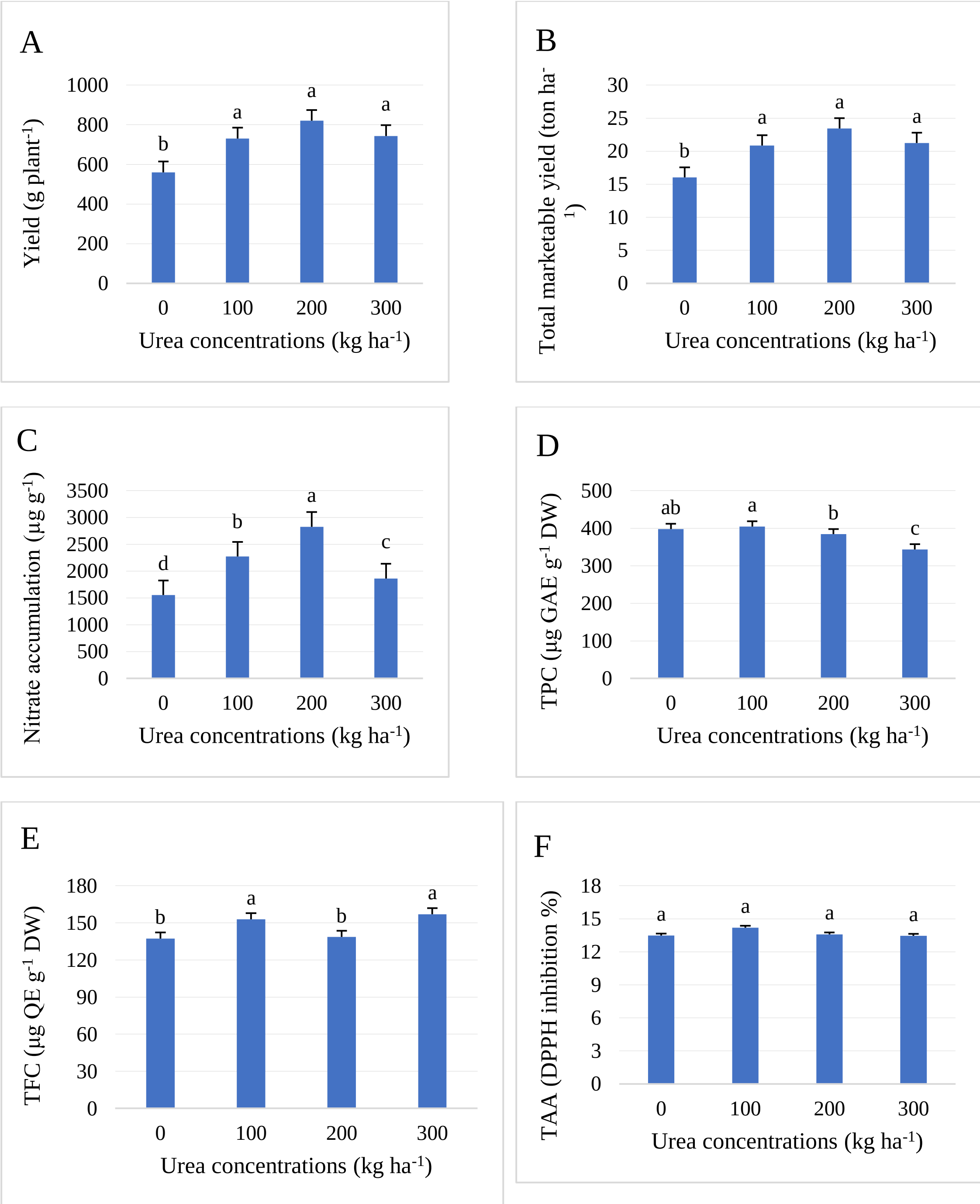
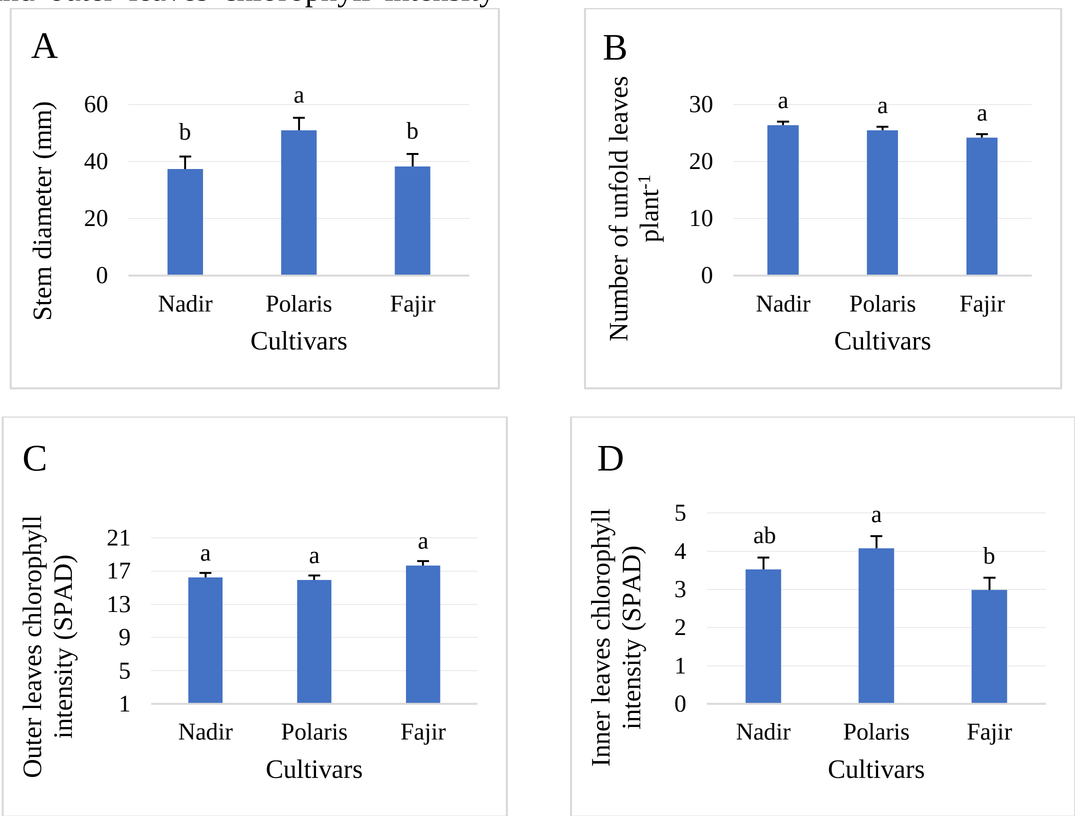


Figure 2. *Effect of urea concentrations on (A) Plant yield, (B)* total marketable yield, *(C) nitrate accumulation (D) total phenolic content (TPC), (E) total flavonoid content (TFC), and (F) total antioxidant activity (TAA) of the lettuce plant. Different letters between columns indicate significant differences at (P < 0.05).*

Effect of lettuce cultivars on the studied variables

The impact of lettuce cultivars on stem diameter, number of unfolded leaves per plant, outer and inner leaves chlorophyll intensity, leaves dry matter percentage, and leaves TSS are shown in (Figure 3). The Polaris cultivar had the thickest stem (51.03 mm) which was significantly different from Nadir and Fajir cultivars by 36.3 and 33.1%, respectively. In addition, Nadir and Fajir cultivars were not statistically different between them in this character (Figure 3A). Furthermore, no substantial variations were observed among the cultivars in number of unfolded leaves per plant (Figure 3B) and outer leaves chlorophyll intensity (Figure 3C). At the same time, the Polaris cultivar recorded the highest chlorophyll intensity in the inner leaves (4.08 SPAD) which was not different from Nadir, while it was significantly superior to the Fajir cultivar by 36.5% (Figure 3D). Furthermore, the Polaris cultivar had the highest leaves dry matter percentage (3.65%) which was significantly different with Nadir and Fajir cultivars by 15.9 and 23.3%, respectively. Also, both Nadir and Fajir cultivars were not statistically different in this character (Figure 3E). In addition, no significant differences were observed among the cultivars in the leaves TSS (Figure 3F).



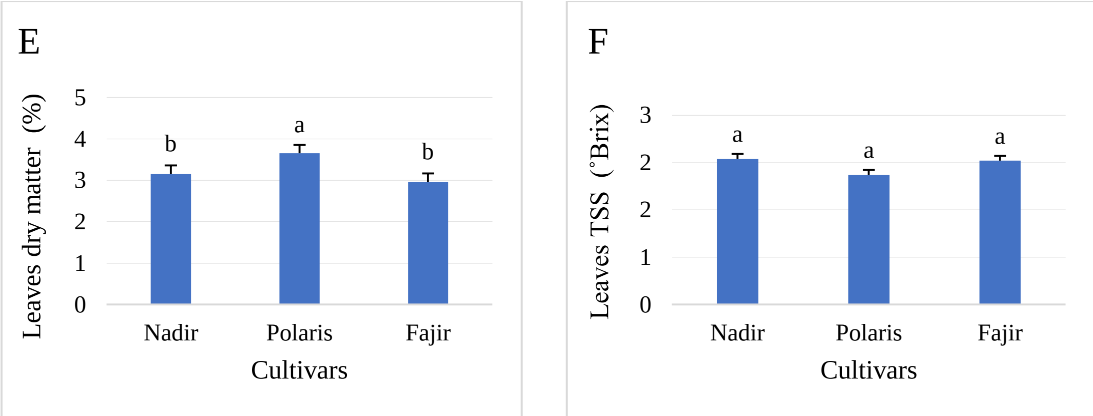
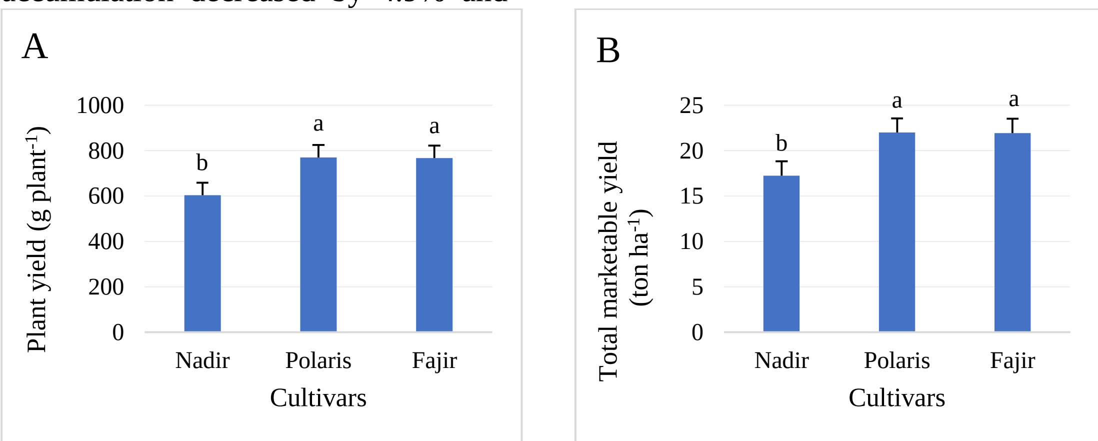


Figure 3. *Effect of lettuce cultivars on (A) stem diameter, (B) number of unfold leaves per plant, (C) outer leaves chlorophyll intensity (D) inner leaves chlorophyll intensity, (E) leaves dry matter, and (F) leaves TSS. Different letters between columns indicate significant differences at (P < 0.05).*

The influence of different lettuce cultivars on yield, nitrate accumulation and phytochemical contents in the leaves are showed in (Figure 4). The yield of the Polaris and Fajir cultivars was not different significantly; and they have given the highest head weight (769.58 and 767.50 g, respectively). These cultivars were significantly superior to Nadir cultivar by 27.5 and 27.2%, respectively (Figure 4A). A similar tendency was observed in the total marketable yield (Figure 4B). In addition, Polaris cultivar accumulated the least amount of nitrate in its leaves (1911.08 µg g^{-1}) which compared to Nadir cultivar its accumulation decreased by 4.9% and compared to Fajir cultivar decreased by 22.8% (Figure 4C). Regarding antioxidants, the Nadir cultivar contained the highest TPC and TFC in the leaves (448.00 µg GAE g^{-1} DW and 169.75 µg QE g^{-1} DW, respectively). The TPC in this cultivar was significantly higher than Polaris and Fajir cultivars by 26.7 and 29.1% respectively (Figure 4D), as well as by 38.9 and 15.2% concerning the TFC (Figure 4E). Additionally, the Fajir cultivar recorded maximum inhibition percentage of the DPPH radical (15.26%) which was more active than Polaris and Nadir cultivars by 30.8 and 8.2%, respectively (Figure 4F).



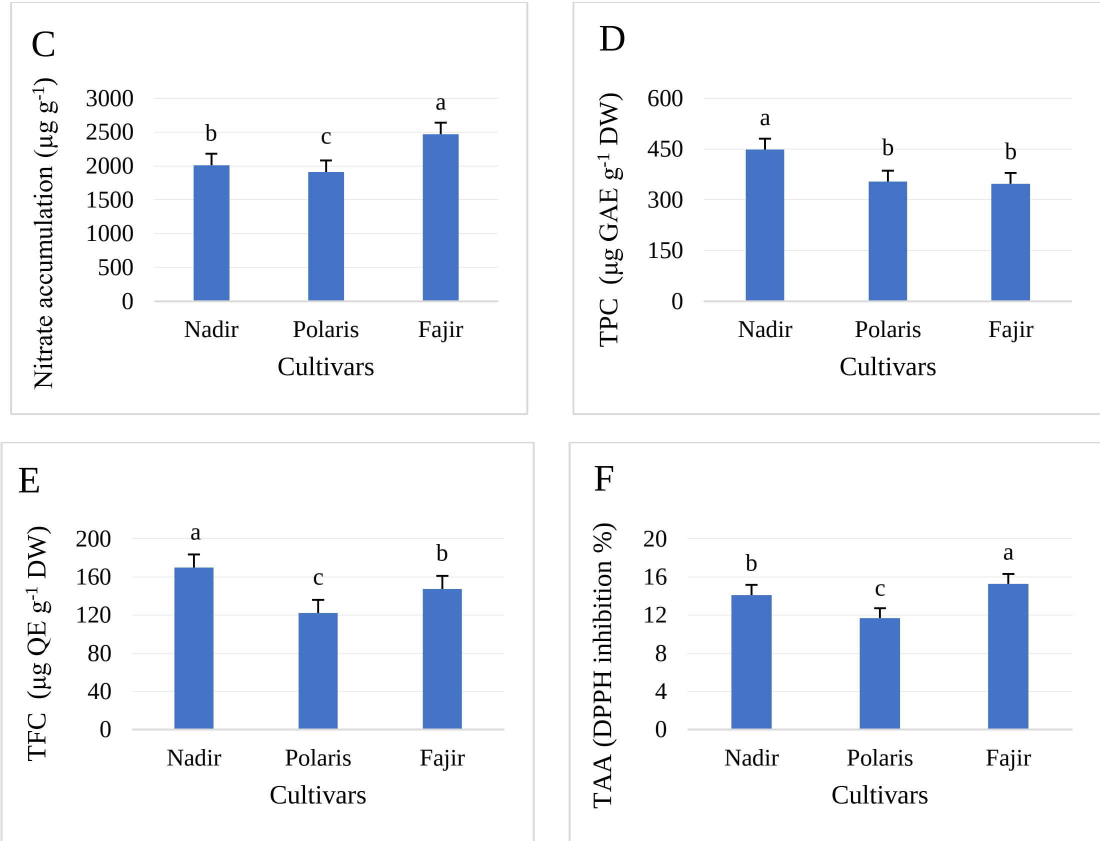


Figure 4. *Effect of lettuce cultivars on (A) plant yield, (B)* total marketable yield, *(C) nitrate accumulation (D) total phenolic content (TPC), (E) total flavonoid content (TFC), and (F) total antioxidant activity (TAA). Different letters between columns indicate significant differences at (P < 0.05).*

Effect of interaction between lettuce cultivars and urea supplements on the studied variables

As shown in Table 2, there are significant differences among some interactions between the doses of urea supplements and the tested lettuce cultivars in terms of stem diameter, the number of unfolded leaves per plant, chlorophyll intensity in the outer and inner leaves, dry matter percentage, and leaves TSS. The maximum values of stem diameter (53.10 mm), and the number of unfolded leaves per plant (27.67) were found in the interaction between the Polaris cultivar and 300 kg ha^{-1} urea. While, Fajir cultivar without application of urea fertilizer resulted in the minimum stem diameter (34.04 mm) and number of unfolded leaves per plant (22.00). Moreover, the highest chlorophyll intensity of the outer leaves (19.67 SPAD) was existed in the Fajir cultivar supplied with 200 kg ha^{-1} urea, the lowest value (12.33 SPAD) was observed in the Polaris cultivar without urea supplement. Additionally, the Polaris cultivar provided with 300 kg ha^{-1} urea had the highest chlorophyll intensity of the inner leaves (4.63 SPAD), whereas the combination between Fajir cultivar with 100 kg ha^{-1} urea had the lowest value (2.60 SPAD). Furthermore, the highest leaves dry matter percentage (4.29%) and the lowest percentage (2.54) were registered by Polaris and Fajir cultivars without urea addition, respectively. Regarding the leaves TSS, interaction of Fajir cultivar with 300 kg ha^{-1} urea exhibited the highest TSS (3.10 %), while Polaris cultivar at 200 kg ha^{-1} urea showed the lowest TSS (1.92%).

Table 2. Interaction effects between lettuce cultivars and urea supplements on stem diameter, number of unfolded leaves per plant, chlorophyll intensity, leaves dry matter percentage and TSS.

Cultivars	Urea Concentrations (kg ha^{-1})	Stem Diameter (mm)	Number of unfold leaves plant^{-1}	Outer leaves chlorophyll intensity (SPAD)	Inner leaves chlorophyll intensity (SPAD)	Leaves dry matter (%)	Leaves TSS (˚Brix)
Nadir	0	35.82 c	25.33 ab	14.83 ab	2.90 bc	2.82 b	2.35 bcd
	100	37.05 c	27.00 ab	17.00 ab	3.17 bc	2.82 b	2.57 abc
	200	38.37 c	27.50 a	14.83 ab	3.77 abc	2.87 b	2.28 bcd
	300	38.58 c	25.67 ab	18.33 a	4.23 ab	4.10 a	2.63 abc
Polaris	0	46.77 b	24.33 ab	12.33 b	3.50 abc	4.29 a	2.67 ab
	100	52.18 ab	25.17 ab	17.33 a	3.63 abc	3.79 a	2.17 bcd
	200	52.07 ab	24.83 ab	16.67 ab	4.57 a	3.70 a	1.92 d
	300	53.10 a	27.67 a	17.33 a	4.63 a	2.82 b	2.02 cd
Fajir	0	34.04 c	22.00 b	15.33 ab	3.10 bc	2.54 b	2.48 abcd
	100	38.87 c	24.50 ab	17.17 ab	2.60 c	2.71 b	2.10 bcd
	200	40.08 c	24.67 ab	19.67 a	2.93 bc	2.89 b	2.05 bcd
	300	40.42 c	25.67 ab	18.50 a	3.33 abc	3.71 a	3.10 a

Different letters between columns indicate significant differences at ($P < 0.05$).

Significant differences in yield, nitrate accumulation and phytochemical characteristics are found in the combination impacts between the lettuce cultivars and urea supplement doses (Table 3). Although the maximum yield (870.83 g plant^{-1}) and total marketable yield (24.88 ton ha^{-1}) were produced by Fajir cultivar combined with 300 kg ha^{-1} urea, but the Fajir and Polaris cultivars fertilized with 200 kg ha^{-1} urea were not different with it which recorded (865.83 and 860.00 g plant^{-1}) and (24.74 and 24.57 ton ha^{-1}) respectively.

The minimum values of plant yield (506.67 g plant^{-1}) and total marketable (14.48 ton ha^{-1}) were obtained in the Nadir cultivar without urea supplement. The lowest nitrate accumulation (1259.67 µg g^{-1}) was recorded by Polaris cultivar without urea application, while, the highest (3896.67 µg.g^{-1}) was found in Fajir cultivar interacted with 200 kg ha^{-1} urea. Concerning the phytochemical contents, the highest content of TPC (569.25 µg GAE g^{-1} DW) was recorded by the Nadir cultivar without urea supplement. While, the lowest TPC

(240.29 µg GAE g^{-1} DW) was registered by Fajir cultivar fertilized with 300 kg ha^{-1} urea. Additionally, the Nadir cultivar and 100 kg ha^{-1} urea gave the maximum content TFC (195.27 µg QE g^{-1} DW), but the minimum content (108.02 µg QE g^{-1} DW) was recorded by the interaction of Polaris cultivar and 100 kg ha^{-1} urea. Moreover, it was found that the interactions between Fajir cultivar and 100 kg ha^{-1} urea registered the highest antioxidant activity for inhibition percentage of DPPH radical (15.99 %), whereas the interaction of Polaris cultivar and 300 kg ha^{-1} urea gave the minimum inhibition percentage (11.36 %).

Table 3. Interaction effects between lettuce cultivars and urea supplements on yield, total marketable yield, nitrate accumulation, TPC, TFC and TAA

Cultivars	Urea Concentrations (kg ha^{-1})	Yield (g $plant^{-1}$)	Total marketable yield (ton ha^{-1})	Nitrate (µg g^{-1})	TPC (µg GAE g^{-1} DW)	TFC (µg QE g^{-1} DW)	TAA (DPPH inhibition %)
Nadir	0	506.67 d	14.48 d	1714.67 h	569.25 a	141.77 cde	13.15 bcd
	100	572.50 bcd	16.36 bcd	2214.00 e	486.35 b	195.27 a	15.15 ab
	200	734.17 abcd	20.98 abcd	2266.67 d	421.19 c	152.74 bcd	14.91 ab
	300	600.83 abcd	17.17 abcd	1844.00 g	315.19 fg	189.23 a	13.18 bcd
Polaris	0	627.50 abcd	17.93 abcd	1259.67 j	285.73 h	129.56 efg	12.36 cd
	100	832.50 ab	23.78 ab	2218.00 e	305.08 gh	108.02 h	11.47 d
	200	860.00 a	24.57 a	2318.67 c	347.15 e	126.41 fg	11.50 d
	300	758.33 abcd	21.67 abcd	1848.00 g	475.99 b	124.90 g	11.36 d
Fajir	0	546.67 cd	15.62 cd	1684.33 i	339.29 ef	140.67 def	14.89 ab
	100	786.67 abc	22.48 abc	2384.67 b	423.18 c	155.35 bc	15.99 a
	200	865.83 a	24.74 a	3896.67 a	384.86 d	136.56 efg	14.36 abc
	300	870.83 a	24.88 a	1903.33 f	240.29 i	156.86 b	15.80

Different letters between columns indicate significant differences at ($P < 0.05$).

Principle Component Analysis (PCA)

The PCA was applied to investigate the relationship between the urea supplementations and the studied variables. The first two main components (PC1 and PC2) together explained 86.87% of the observed variation and were thus represented in two dimensions (Figure 5). PC1, plotted on the horizontal axis, illustrated the highest proportion of the variance (54.23%), while PC2, plotted on the vertical axis, accounted for a further 32.64% of the total variation. The PCA results showed that the urea treatment strongly impacts the studied variables. So, the 300 kg ha^{-1} application significantly impacted the NUL, OCI, ICI, DM and TFC. In addition, 100 and 200 kg ha^{-1} significantly affected SD, Y, TMY, Nit and TAA. However, the TSS and TPC were slightly influenced by urea applications.

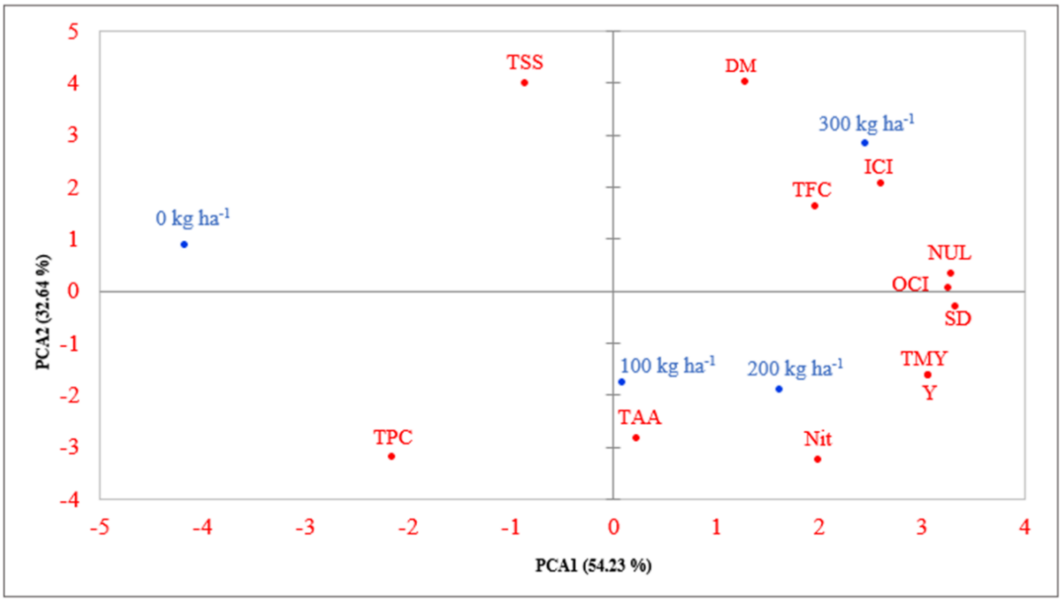


Figure 5. PCA biplot illustrating the distributions of the urea supplement levels (0, 100, 200 and 300 kg ha^{-1}) depending on the studied variables: plant yield (Y), total marketable yield (TMY), stem diameter (SD), number of unfold leaves per plant (NUL), outer leaves chlorophyll intensity (OCI), inner leaves chlorophyll intensity (ICI), dry matter (DM), leaves TSS, nitrate (Nit), total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant activity (TAA).

Also, the PCA was conducted to determine the relationship between the cultivars and the variables. The first two main components (PC1 and PC2) together explained 100% of the observed variation and were thus represented in two dimensions (Figure 6). PC1, plotted on the horizontal axis, illustrated the highest proportion of the variance (57.82%), while PC2, plotted on the vertical axis, accounted for a further 42.18% of the total variation. The PCA results showed that there was clear variation among all the cultivars. Polaris cultivar was distinctively varied from other two cultivars (Nadir and Fajer). In addition, the Polaris cultivar showed positive effect on SD, NUL, ICI, DM, Y and TMY variables. However, Nadir showed close association with TSS, TPC and TFC, while Fajir showed coloration with affected the OCI, Nit and TAA.

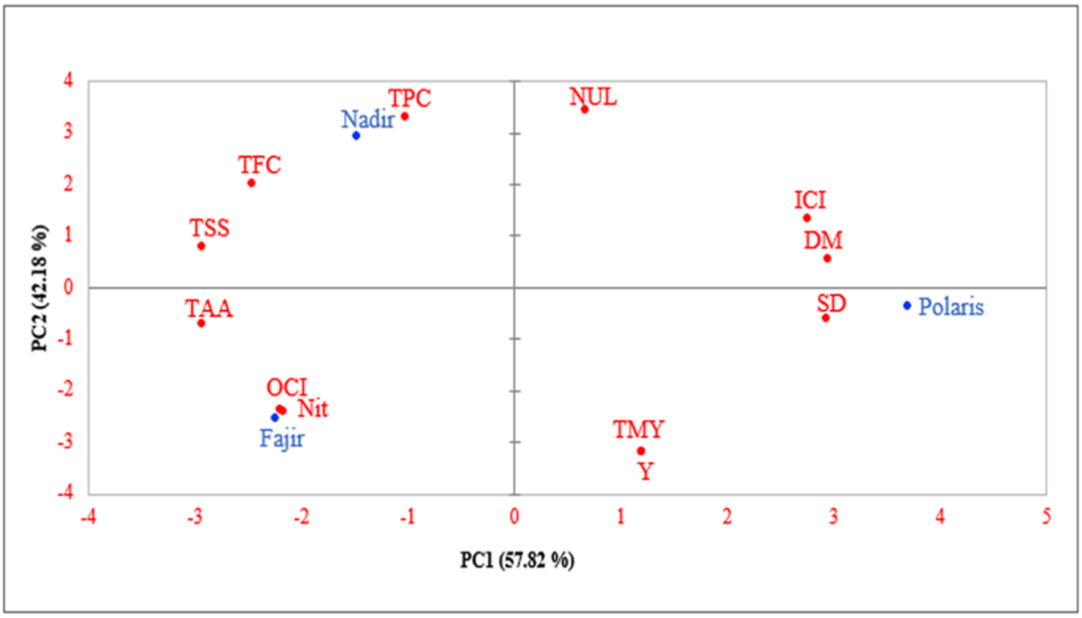


Figure 6. PCA biplot illustrating the distributions of the investigated cultivars (Nadir, Polaris and Fajir) of lettuce plant depended on the studied variables: plant yield (Y), total marketable yield (TMY), stem diameter (SD), number of unfold leaves per plant (NUL), outer leaves chlorophyll intensity (OCI), inner leaves chlorophyll intensity (ICI), dry matter (DM), leaves TSS, nitrate (Nit), total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant activity (TAA).

Multivariate Analysis

Pearson's correlation was conducted to reveal the association between the studied variables (Figure 7). The results showed that there was a strong positive correlation between stem diameter and the plant yield as well as total makeable yield (r^2 = 0.58 at $p <$ 0.001), and also with inner leaves chlorophyll intensity (r^2 = 0.42 at $p <$ 0.01). However, significant negative links were observed between stem diameter and total antioxidants activity (r^2 = -0.58 at $p <$ 0.001), total flavonoids content (r^2 = -0.60 at $p <$ 0.001), and TSS (r^2 = -0.37 at $p <$ 0.05). In addition to the stem diameter, the plant yield and total marketable yield increased significantly when nitrate concentration increased (r^2= 0.42 at $p <$ 0.05) while they decreased significantly when TSS increased (r^2 = -0.43 at $p <$ 0.001). Additionally, the total marketable yield is strongly and positively associated with plant yield (r^2 = 1 at $p <$ 0.001). Furthermore, nitrate concentration significantly and positively correlated with outer leaves chlorophyll intensity (r^2 = 0.45 at $p <$ 0.001), whereas it showed a negative correlation with TSS (r^2 = -0.37 at $p <$ 0.05). The total phenolic content showed a negative relationship with both dry matter (r^2= -0.60 at $p <$ 0.001) and TSS (r^2= -0.34 at $p <$ 0.05). Finally, there was a strong positive relationship between total flavonoids content and total antioxidants activity (r^2 = 0.46 at $p <$ 0.001).

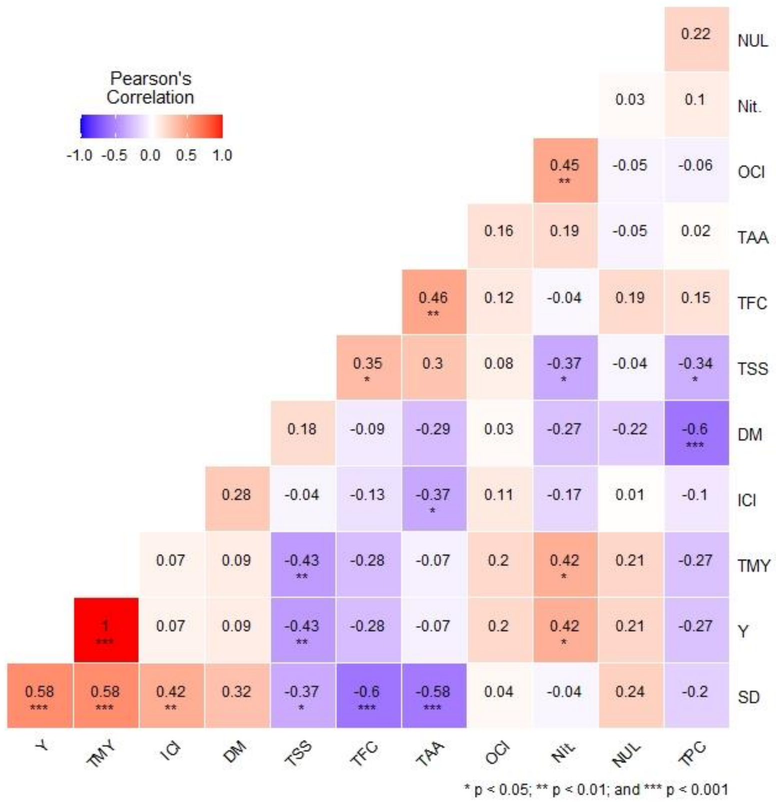


Figure 7. Pearson's correlation between all the studied variables supported with correlation direction and significant threshold level.

Discussion

Different factors impact the vegetable production. Urea supplements and selecting the suitable cultivars directly contribute in leafy, especially lettuce, vegetables quality and quantity such as yield and yield components, nitrate accumulation, and various physiochemical properties.

Urea supplements, as an N source, notably impact the nitrate accumulation as well as the lettuce quantity and quality (Figures 1 and 2). [22] and [23] explained that increasing the amount of nitrogen fertilizer has a positive effect on the dry weight of leaves due to increments in leaf surface caused by increased plant development and resulting in higher photosynthetic capacity; consequently, the amount of dry matter increased and helped micronutrients to be transported from the root to the shoot system. Therefore, a significant and positive correlation was observed between nitrate and both yield and marketable yield (Figure 7). In contrast, studies showed that the concentration of nitrate decreased significantly when TSS increased [24]. [25] stated that increasing the N application rate resulted in an increase in fresh weight, nitrate, K, Mg, and Mn content in lettuce. Furthermore, studies reported that nitrogen fertilizer application (120 kg ha^{-1}) significantly raised the yield of lettuce, while the yield decreased markedly at the higher nitrogen level, which might be due to the toxicity reaction of plants or an imbalance between nutrients in the plant [24] and [26]. From another point of view, it appears that nitrate accumulated more in the older and outer leaves of lettuce than in the inner leaves [27]. Thereby, implementing a high amount of fertilizer for plants is not necessary because their assimilation is dependent on leaf growth, which relies on other factors [28].

Alongside with the urea supplement, our findings emphasized that the cultivars significantly impacted the overall yield; in addition, they showed different responses to nitrate accumulation (Figures 3 and 4). Many studies also reported similar results; [29] found that lettuce plants at marketable maturity depend on cultivars. Additionally, research has demonstrated that the type of cultivar of vegetables has an important role in nitrate content, and the influence of cultivar on nitrate accumulation has been determined in investigations frequently. Moreover, explained that accumulation of nitrate in lettuce plants was partially related to the variations that occurred among the cultivars [9]; [30] and [31]. In addition, the interaction between cultivars and urea supplement significantly affects the nitrate accumulation, yield, and yield component characteristics [32].

Conclusions

Nitrogen is an essential nutrient element for plant growth and development. However, nitrate accumulation, especially in leafy vegetables, seriously impacts human health. The results of this study showed that the urea supplements and cultivars significantly influenced the nitrate accumulation as well as various lettuce quality and quantity characteristics. The nitrate accumulation increased when 100 and 200 kg ha^{-1} were applied but decreased when 300 kg ha^{-1} was applied. The Polaris cultivar gave the best results in some traits. The interaction between the Polaris cultivar and 200 kg ha^{-1} urea maintained a good balance between the yield and its quality. The findings of this study will be useful in informing lettuce producers about the misuse of urea supplements under greenhouse circumstances.

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مجلة جامعة كركوك للعلوم الزراعية

تأثير مكملات اليوريا و الاصناف على الحاصل و تراكم النترات و بعض صفات الكيميائية على الخس تحت ظروف البيوت المحمية

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• تاريخ استلام البحث 31/03/2023 وتاريخ قبوله 24/04/2023

المستخلص

تم تقييم تأثير مكملات اليوريا 100 و 200 و 300 كغم هكتار$^{-1}$ بالإضافة الى معاملة القياس و هجن الخس نادر و بولاريس وفجر على الحاصل وتراكم النترات وبعض الصفات الكيميائية تحت ظروف البيوت البلاستيكية. بينت النتائج أن جميع معاملات اليوريا التكميلية أدت إلى زيادة معنوية في قطر الساق وكثافة الكلوروفيل في الأوراق الخارجية وحاصل النبات و إجمالي الحاصل التسويقي. بالإضافة إلى ذلك، زادت جرعات اليوريا بشكل ملحوظ من تراكم النترات في الأوراق، بينما انخفض تراكمها عند أعلى مستوى جرعة مقارنة بالجرعات الأخرى. علاوة على ذلك، انخفض TPC بشكل معنوي عند اضافة جرعة عالية من مكمل اليوريا. بالإضافة إلى ذلك، فإن مكمل اليوريا، وخاصة 100 و 300 كغم هكتار$^{-1}$، أدى إلى زيادة TFC بشكل ملحوظ. أظهر الصنف بولاريس أعلى قيمة لقطر الساق، شدة الكلوروفيل في الأوراق الداخلية، نسبة المادة الجافة، حاصل النبات، إجمالي الحاصل التسويقي بالإضافة إلى الحد الأدنى من النترات المتراكم في الأوراق. علاوة على ذلك، حصل الصنف نادر على أعلى محتوى من TPC و TFC. بالإضافة الى ذلك سجل صنف الفجر اعلى نشاط لمضادات الأكسدة الكلية. أخيرا، حافظ معاملة التداخل بين صنف بولاريس و 200 كغم هكتار$^{-1}$ من اليوريا على توازن جيد بين الحاصل وجودته. نتيجة لذلك، قد تساعد نتائج هذه الدراسة مزارعينا على تبني تراكيز اليوريا المثلى مع أصناف الخس في حقولهم تحت ظروف البيوت البلاستيكية.

الكلمات المفتاحية: *Lactuca sativa* L.، الانتاجية، مضادات الاكسدة، TPC, TFC, DPPH, NO_3