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Effect of growth seasons and nitrogen fertilization on the growth, yield and nitrate accumulation of lettuce (Lactuca sativa L.) plants

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***Abstract*---**In modern agriculture, the nitrate accumulation in vegetables has become a serious threat to human health. That caused by an imbalance between nitrate absorption and assimilation in plants. Nitrogen fertilization and light intensity are the main factors affected nitrate accumulation. Therefore, a pot experiment was conducted during two successive summer and winter seasons of 2019/20 at the greenhouse of Fac. Agric., Cairo Univ., Giza, Egypt, on lettuce (Lactuca sativa L. var. longifolia Lam) plants to investigate the effect of growth seasons (summer and winter), different rates (0, 60, 90 and 120 Kg N fed^{-1}) and sources (CO $(NH_2)_2$, NH_4NO_3 and $(NH_4)_2SO_4$) of nitrogen fertilizers on lettuce growth and nitrate concentrations. The results showed that fresh and dry weight, yield and nutrient contents of lettuce plants increased gradually according to increasing dose of nitrogen, up to 90 Kg N fed^{-1} and the magnitude of increase, according to different N fertilizers used , was in the following order: CO $(NH_2)_2$ > NH_4NO_3 > $(NH_4)_2SO_4$. The accumulation of nitrate was higher in winter compared to summer season for

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both inner and outer leaves. The highest values for both seasons were recorded in the outer leaves, which increased significantly with increasing N fertilizer dose and the magnitude of increase, according to N sources used, was in the following order: NH_4NO_3 > $CO(NH_2)_2$ > $(NH_4)_2SO_4$. This study recommend the combined use of urea form at 90 Kg N fed^{-1} in summer season to achieve the highest yield with acceptable nitrate content.

***Keywords*---**nitrogen fertilizer, lettuce, nitrate, growth seasons.

Introduction

Lettuce (Lactuca sativa L.) is the most consumed leafy vegetable in the human diet. It is a good source of minerals, vitamins, fiber, and proteins (Kim et al., 2016; Mampholo et al., 2016; Das and Bhattacharjee, 2020) as well as antioxidant that are very important to reduce the risk of cancer and cardiovascular disease (Viacava et al., 2014; Mohankumar et al., 2018). However, the accumulation of nitrate is considered a serious threat to the environment and human health (Ortega-Blu, et al., 2020). Lettuce needs a high dose of N to give economical yield and low N supply led to decrease the yield (Saah et al., 2022). It contains high levels (over 2500 mg kg^{-1} fresh weight) of nitrate (Santamaria, 2006) and the European Commission (European Commission Regulation (EU) No 1258/2011) has set maximum level for nitrate in summer/winter lettuce grown under cover at 4000/5000 mg kg^{-1} FW (Jokinen et al., 2022).

Accumulation of NO_3-N in plant tissue is resulting from higher absorption compared to its assimilation of this ion in plants (Chowdhury and Das, 2013). Which is related to many biological, environmental and agricultural factors (Qiu et al., 2014). The main factors which influenced NO_3 accumulation in plants are light intensity and N fertilization (Santamaria, 2006). Light is one of the most important factors regulating N assimilation, not only provides energy for drivingphotosynthesis but also light enhances nitrate reduction by increasing the activity of nitrate reductase (NR) enzyme (Iglesias-Bartolomé, 2004), which decreases under low light conditions, leading to an accumulation of nitrate (Nazaryuk et al., 2002). Ortega-Blu, et al., (2020) reported that N assimilation was higher significantly in summer compared to the winter season; therefore NO_3 accumulation in lettuce plants was higher in winter than in summer season.

Nitrate accumulation in vegetables could be immediately linked to N fertilization practices, excess nitrogen applied resulted in increased NO_3 accumulation (Ortega-Blu et al., 2020). Various studies reported a significant positive correlation between NO_3 content in leafy vegetables and the dose of nitrogen supply (Fu et al., 2017; Urlić et al., 2017). Also nitrate accumulation can be related to the form of N-fertilizer (Pavlou et al., 2007). Nitrate accumulation can be reduced by growing the plants in a nitrate-free or nitrate-limited solution for a period before harvest, replacing the nitrate with other forms of nitrogen such as ammonium-based nitrogenous fertilizers (Ahmed et al., 2020; Wang and Li, 2004), using nitrate inhibitors, or modifying the light intensity and its quality and photoperiod (Colla et al., 2018). Renseigné et al., (2007), also reported that the

NO_3 accumulation in the following order: $CO(NH_2)_2$ > NH_4NO_3 > $(NH_4)_2SO_4$. Finally, it is certainly necessary to adapt the correct management of N fertilization through application right N fertilizer form at optimum rate and application time. The aim of this study is to produce healthy lettuce with high yield and low nitrate content. Therefore, a pot experiment was conducted during summer and winter seasons using varying sources of nitrogen fertilizers with different doses under both growth seasons to select the best N management practices in combination with growth season that produce the highest lettuce yield with acceptable NO_3 accumulation.

Materials and Methods

Soil Sampling and Analysis

Pot trial was conducted during two successive growth seasons (summer and winter) of 2019 at the greenhouse in Agricultural Cairo University, Giza, Egypt. Soil sample were collected from the Faculty of Agriculture farm, Cairo University (latitude: 31°35'56" N; longitude: 31°21' E; mean altitude: 5 m above sea level). Prior to starting the experiment, soil sample were taken from the experimental sites and analyzed for their some physical and chemical properties (Table 1), following the methods described by Chapman and Pratt, (1978).

Table 1. Some physico-chemical properties of soil used

Particle Size Distribution			Soil Texture	pH 1:2.5	ECe dSm^{-1}	Soluble Cations(meq l^{-1})				Soluble Anions(meql^{-1})				Available Macronutrients (mg Kg^{-1})		
Sand (%)	Silt (%)	Clay (%)				Na^+	K^+	Ca^{++}	Mg^{++}	HCO_3^-	Cl^-	SO_4^{--}	CO_3^{--}	N	P	K
27.17	35.23	37.60	Clay loam	7.86	1.88	4.55	0.85	7.66	5.74	2.54	12.21	3.6	0.45	28.00	3.20	85.00

Experimental Design and Treatments

Factorial experiment (2 X 4 X 3) was carried out in a randomized complete block design (RCBD) with three replications for each treatment. The experiment consisted of three factors: factor A: two growth seasons: summer (14-8-2019/20) and winter (15-12-2019/20). Factor B: four nitrogen levels (N0=no N addition as control, N1=60, N2=90 and N3=120 kg N fed^{-1}). Factor C: three nitrogen sources (Urea (U.) 46% N, ammonium nitrate (A.N.) 33.5% N and ammonium sulphate (A.S.) 21% N).

Experimental Procedure

A plastic pot with a capacity of about 10 kg of soil was used. The soil was prepared before planting, phosphate and potassium fertilizers were applied in accordance with the fertilizer amounts recommended by the Ministry of Agriculture. After germination six seedlings of lettuce (*Lactuca sativa* L. var. *longifolia* Lam) were transplanted in each pot and thinned to three seedlings after emergence. Then after a week, half of the used N rates from different N forms were added to the matching pots as N fertilizer solution and the other dose was added two weeks later.

Data Recording

At harvest (6 weeks after transplanting) all heads were cut at the soil surface, and plant fresh weights were recorded. The leaves of harvested plants were separated into outer (green) and inner (pale yellow) leaves, washed in tap water and dried by oven on 70 °C then the plant dry weights were recorded.

Chemical analysis

Determinations of total nitrogen according to the Kjeldahl method (Keeney and Nelson, 1982). Potassium was determined by emission flame photometer (Jackson, 1958). Phosphorus was determined colorimetrically using the molybdephosphoric blue colour method in sulphuric acid system as described by Jackson (1958). Nitrogen present as nitrate was determined into outer and inner leaves according to the method described by Singh (1988).

Statistical Analyses

Test of normality distribution was carried out according to Shapiro and Wick, method (1965), by using SPSS v. 17.0 (2008) computer package. A randomized complete block design with three factors were used for analysis all data with three replications for each parameter. Estimates of LSD were calculated to test the significance of differences among means according to Seducer and Cochran (1994) by used assistant program.

Results and Discussion

Fresh, dry weight and yield of lettuce head

Results in Table 2 showed that, a significant difference (at $p \leq 0.05$) between winter and summer growth seasons for fresh, dry weight and head yield of lettuce. The highest fresh weight and yield of lettuce head were obtained in the winter growth season, on the opposite the heights dry weight of lettuce head was obtained in the summer growth season that may be due to the moisture content which higher in winter lettuce. In this respect Fu et al., (2017) reported that an increase in light intensity (summer season) lead to decrease the moisture content of the lettuce. The growth and production of lettuce largely depends on the environment, especially solar radiation. Adams, (2002), reported that the highest yield was obtained in summer, due to the higher solar radiation and longer days, which lead to enhance photosynthesis (Dapoigny et al., 2000). In this respect (Pavlou et al., 2007) also demonstrated that the highest lettuce production was observed in the late-spring season due to extended periods of sunshine.

A significant increase at $p \le 0.05$ for both fresh, dry weight and yield of lettuce plants at all N rates used relative to the control plants (zero N addition), irrespective of the growth seasons and nitrogen source treatments. This result agree with (Awaad et al., 2016), that N application enhances leaf surface area and plant growth which stimulate photosynthetic rate and therefore increased yield (Tei et al., 2000). Results also reported that 90 Kg N fed^{-1} gives the greatest fresh, dry weights and heads yield and decreased with 120 Kg N fed^{-1} while, it was still superior to zero N addition. Fu et al., 2017 reported that the ideal nitrogen fertilizer rate enhances plant growth, but surplus N fertilizer did not achieve the maximum yield. Which may be due to that the excess nitrogen fertilizer resulted in an imbalance of C/N ratio (Martínez-Lüscher et al., 2015) and negatively affects the photosynthetic rate in plant leaves (Zhou et al., 2006). El-Bassyouni, (2016) found that the productivity of lettuce was enhancing with increasing N levels from 60 to 100 kg N fed^{-1}, however, 80 kg N fed^{-1} resulted in the highest lettuce yield, with less accumulation of nitrate relative to the highest rate. Hamdi et al., (2014) reported that increasing N fertilizer up to the optimal dose reduced lettuce head diameter and therefore fresh weight and yield.

The results presented in Table 2 also showed that, irrespective of the growth seasons and nitrogen levels, there were significant differences (at $p \le 0.05$) for fresh, dry weights and heads yield of lettuce plants between all three nitrogen sources used. Which increased significantly, according to nitrogen sources used, in the following order: $CO(NH_2)_2$ > NH_4NO_3 > $(NH_4)_2SO_4$. Similarly, Topcuoglu and Yalcin (1997) reported that application of various forms of nitrogen fertilizers into greenhouse soil resulted in varying effects on the lettuce yield; urea fertilizer gave the highest yield.

In this respect, Marschner (1986) reported that, ammonium may be suppressing the absorption of cations which can inhibit growth by causing magnesium or calcium deficiency. Abd-Elmoniem et al., (1996) observed that plant growth increased, according to nitrogen fertilizer source, in the following order: calcium and potassium nitrate> ammonuim nitrate> urea> ammonuim sulphate. They also reported that the NH_4- fed plants were small with very dark green leaves and a very poorly developed stubby root system compared to NO_3-fed plants. For the interaction effects, it was noted that the highest values of fresh, dry weight and yield of lettuce were obtained with 90 kg N fed^{-1} as urea N fertilizer form under both summer or winter growth season.

Table 2. Effect of different growth seasons and nitrogen treatments on fresh and dry weights (g pot⁻¹) and yield (ton fed⁻¹) of lettuce plants

Seasons	N-Treatments	Shoot F.W. (g pot^{-1})				Shoot D.W. (g pot^{-1})				Yield (ton fed^{-1})			
		U.	A.N.	A.S.	Mean	U.	A.N.	A.S.	Mean	U.	A.N.	A.S.	Mean
Winter	N0	69.65	69.65	69.6	69.65	6.27	6.27	6.27	6.27	5.57	5.57	5.57	5.57
	N1	161.29	86.49	75.84	107.87	15.37	12.91	11.56	13.28	12.90	6.92	6.06	8.63
	N2	195.08	96.88	84.31	125.42	20.76	18.02	13.16	17.31	15.60	7.74	6.74	10.03
	N3	111. 65	78.74	69.53	86.64	12.01	11.24	8.60	10.62	8.93	6.29	5.56	6.93
	Mean	134.42	82.94	74.83	97.40	13.60	12.11	9.89	11.87	10.75	6.63	5.98	7.79
Summer	N0	59.32	59.32	59.32	59.32	6.57	6.57	6.57	6.57	4.74	4.74	4.74	4.74
	N1	150.89	76.50	79.92	102.44	17.28	14.57	11.44	14.43	12.07	6.12	6.39	8.19
	N2	163.99	95.53	90.38	116.63	21.54	18.12	14.77	18.14	13.11	7.64	7.23	9.33
	N3	99.04	74.15	64.12	79.10	11.37	10.56	8.46	10.13	7.92	5.93	5.12	6.32
	Mean	118.31	76.38	73.44	89.37	14.19	12.46	10.31	12.32	9.46	6.11	5.87	7.15
Mean	N0	64.49	64.49	64.49	64.49	6.42	6.42	6.42	6.42	5.15	5.15	5.15	5.15
	N1	156.09	81.50	77.88	105.16	16.33	13.74	11.50	13.86	12.48	6.52	6.23	8.41
	N2	179.54	96.21	87.35	121.03	21.15	18.07	13.97	17.73	14.36	7.69	6.98	9.68
	N3	105.35	76.45	66.83	82.87	11.69	10.90	8.53	10.37	8.42	6.11	5.34	6.63
		126.36	79.66	74.13		13.89	12.28	10.10		10.10	6.37	5.93	
LSD 0.05		A: 1.30, B: 1.84, C: 1.59, A*B: 2.60, A*C: 2.25, B*C:3.19 & A*B*C: 4.5				A: 0.31, B: 0.44, C: 0.53, A*B: 0.62, A*C: 0.54, B*C: 0.76 & A*B*C: 0.87				A: 0.10, B: 0.14, C: 0.17, A*B: 0.20, A*C: 0.18, B*C:0.25 & A*B*C: 0.36			

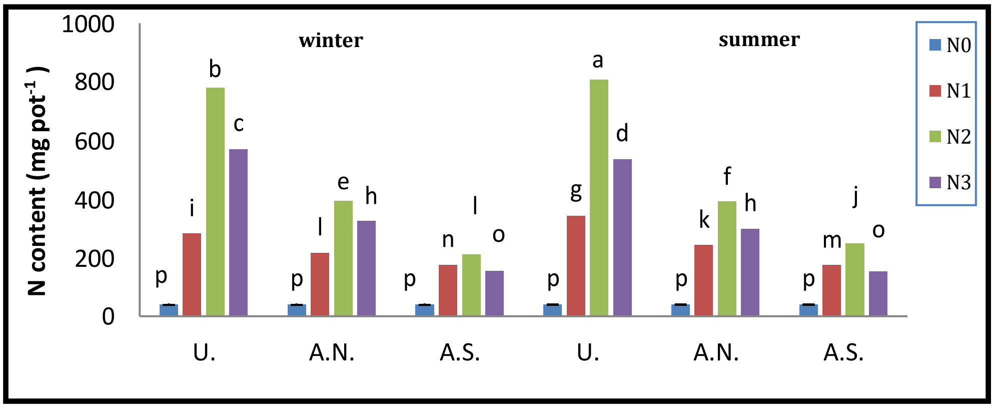
Data are displayed as mean (n = 3). Least Significant Difference (LSD) test at ($p \leq 0.05$) for factor A: growth seasons, factor B: N levels (control N0=no N addition, N1=60, N2=90 and N3=120 kg N fed^{-1}), factor C: N sources (Urea (U.), ammonium nitrate (A.N.) and ammonium sulphate (A.S.)) and the interaction effects: A*B, A*C, B*C & A*B*C.

Nutrient contents in lettuce plants

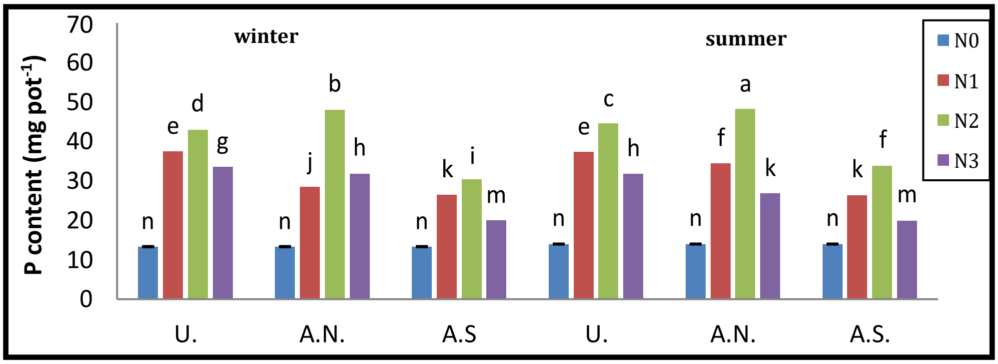
As shown in Figure 1, regardless of the N fertilizer treatments there were no significant differences between winter and summer growth seasons for the contents of P and K in the lettuce plants, while N content was increased significantly (at $p \leq 0.05$) in lettuce plants cultivated in summer season relative to winter season. The application of different levels of N fertilizer increased significantly (at $p \leq 0.05$) nitrogen, phosphorus and potassium contents in the lettuce plants relative to control (no N addition) plants, irrespective of the growth seasons and nitrogen forms. In this respect El-Bassyouni, (2016) reported that with increasing N-fertilizer levels within the range of 60 up to 100 kg N fed^{-1}, nitrogen, phosphorus and potassium contents in lettuce plants were increased. In this regard, the increase in the growth and production of lettuce plants (Table 2) is associated with an increase in nutrient uptake (Fig. 1), which may be due to the enhancement of the photosynthesis rate and thus the stimulation of the growth and productivity of plants. These results are in agreement with those of (El-Ghany et al., 2021; El-Ghany et al., 2022).

Results also showed that, the highest values of N, P and K content in lettuce plants were obtained with 90 kg N fed^{-1} irrespective of, the source of N fertilizer. Similar results were obtained by Awaad et al., (2016); they reported that increasing N fertilizer levels up to 90 kg N fed^{-1} was lead to increase P, K and Ca contents in the lettuce plants. While, increasing the dose of N fertilizer over this rate lead to decrease their contents, but still higher than that of control. N and K contents increased significantly according to nitrogen fertilizer source in the following order: $CO(NH_2)_2 > NH_4NO_3 > (NH_4)_2SO_4$. This variation in N contentmay be due to the differences in both of adsorption and assimilation processes of N forms in the lettuce plants (Abd-Elmoniem et al., 1996).

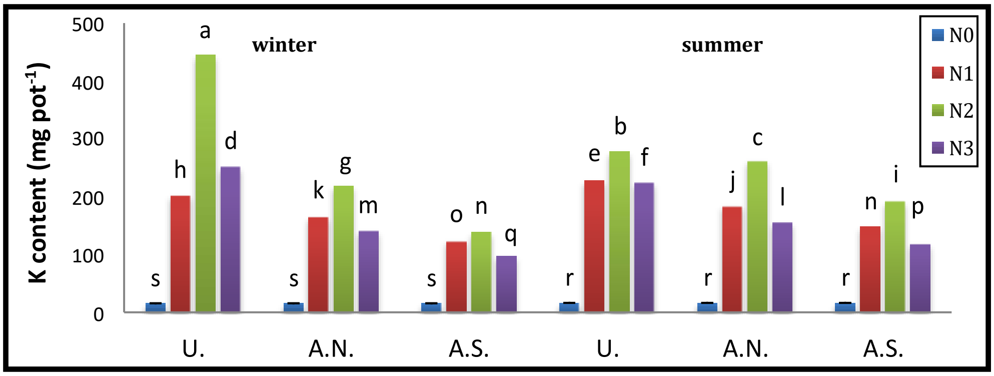
The interaction effect between growth seasons and N fertilization form showed that the highest value of nitrogen (431.81 mg pot^{-1}), phosphorus (31.91 mg pot^{-1}) and potassium (228.74 mg pot^{-1}) contents were attained in lettuce plant treated with urea during summer season for N and P, while in winter season for K. The interaction effect between growth seasons and N fertilization levels results showed that the highest value of N (482.51 mg pot^{-1}), P (42.20 mg pot^{-1}) and K (267.56 mg pot^{-1}) contents were attained in lettuce plant fertilized with 90 kg N fed^{-1} during summer season for N and P, while in winter season for K. The interaction effects between N fertilizer forms and levels results reveal that the highest value of N (793.77 mg pot^{-1}), P (48.05 mg pot^{-1}) and K (361.40 mg pot^{-1}) contents were attained in lettuce plant fertilized with 90 kg N fed^{-1} from urea for N and k, while with ammonium nitrate for P. For the interaction effects between all treatments, that the highest value of N (806.97 mg pot^{-1}) was obtained in lettuce plantstreated with urea at 90 kg N fed^{-1} in summer season, the highest value of P (48.20 mg pot^{-1}) was obtained in plants fertilized with ammonium nitrate at 90 kgN fed^{-1} in summer season and the highest value of K (444.97 mg pot^{-1}) was obtained in plants treated with urea at 90 kg N fed^{-1} in winter season.



(A)



(B)



(C)

Figure 1. Effect of different growth seasons (winter and summer), 4 N levels (control N0=no N addition, N1=60, N2=90 and N3=120 kg N fed^{-1}), and N sources (Urea (U), ammonium nitrate (AN) and ammonium sulphate (AS)) on nutrient contents [A: Nitrogen (N) (mg pot^{-1}), B:Phosphorus (P) (mg pot^{-1}), C: Potassium (K) (mg pot^{-1}), of lettuce plants. The bars of treatment followed by the same letter are not significantly different according to an LSD test ($p \leq 0.05$).

Nitrate accumulation in lettuce

The data shown in Table 3 proved that, outer leaves contained higher quantity of N-NO_3 than inner leaves of lettuce plants at both growth season and at all N treatments. The accumulation of nitrate in outer leaves was 54.49 % higher compared to inner leaves, when lettuce plants cultivated in the winter season and 54.85% higher when plants were grown in the summer season. This result agrees with (Qiu et al., 2014). Cells in outer leaves have large vacuoles (Bensink, 1971) where nitrate can be stored. In this respect Marsic and Osvald (2002) and Hamdi et al., (2014) reported that, the highest NO_3 content was observed in outer leaves, while the lowest ones was obtained in inner leaves of lettuce plants.

Nitrate accumulation in both inner and outer leaves of lettuce cultivated in the winter increased significantly (at $p \leq 0.05$) compared to lettuce cultivated in summer season irrespective of nitrogen treatments. The magnitude of increase in the inner and outer leaves were 24.84% and 23.84%, respectively. These results agree with the results obtained by Ortega-Blu et al., (2020). Iglesias-Bartolomé, (2004) reported that under low light condition (winter season) the activity of nitrate reductase (NR) enzyme decreased, leading to an accumulation of nitrate (Nazaryuk et al., 2002). The application of N fertilizer increased significantly the nitrate contents of inner and outer leaves of lettuce plants compared with the control (Table 3). Nitrate accumulation in inner and outer leaves of lettuce plants increased significantly (at $p \leq 0.05$) as nitrogen fertilizer dose increased, regardless the growth season and nitrogen fertilizer source, this result agrees with Ahmadi et al. (2010). Urlić et al., (2017) and Ortega-Blu et al., (2020) reported that the NO_3-N accumulation in vegetables is linked to N fertilizer especially, the excess of N fertilizer dose.

The results presented in Table 3 also show that, irrespective of the growth seasons and nitrogen levels, there were significant differences (at $p \leq 0.05$) for nitrate accumulation in inner and outer leaves of lettuce plants between all three nitrogen sources used. Nitrate accumulation in both inner and outer leaves of lettuce plants increased, according to applied N form, in the following order: ammonium nitrate > urea > ammonium sulphate. Similarly, Topcuoglu and Yalcin (1997) reported that the lowest NO_3 accumulation was observed in lettuce plants fertilized with $(NH_4)_2SO_4$, where the highest ones was obtained with Ca $(NO_3)_2$.

Similarly, results demonstrated that the highest NO_3 accumulation (2521.32 mg Kg^{-1}) was observed in outer leaves of lettuce plants treated with ammonium nitrate N-fertilizer at 120 Kg N fed^{-1} and cultivated in the winter. While the lowest values of NO_3 accumulation (251.63 and 405.90 mg Kg^{-1}) were obtained in inner leaves of lettuce plants without N addition in summer and winter seasons, respectively. Which reached in outer leaves (413.97 mg Kg^{-1} for zero N addition plants in summer season), followed by (429.33 mg Kg^{-1}) in inner leaves of plants treated with ammonium sulphate N-fertilizer at 60 Kg N fed^{-1} cultivated in the summer.

Table 3. Effect of different growth seasons and nitrogen treatments on nitrate accumulation (mg Kg^{-1}) in inner, outer and total leaves of lettuce plants

Seasons	Ntreat-ments	Nitrate accumulation (mg kg $^{-1}$)							
		Inner				Outer			
		U.	A.N.	A.S.	Mean	U.	A.N.	A.S.	Mean
Winter	N0	405.90	405.90	405.90	405.90	783.70	783.70	783.70	783.70
	N1	655.61	1091.73	540.34	762.56	1612.29	2175.30	1128.80	1638.80
	N2	749.56	1117.06	559.45	808.69	1736.52	2358.80	1429.32	1841.55
	N3	789.06	1158.94	621.32	856.44	1845.35	2521.32	1518.09	1961.59
	Mean	650.03	943.41	531.75	708.40	1494.47	1959.78	1214.98	1556.41
summer	N0	251.63	251.63	251.63	251.63	413.97	413.97	413.97	413.97
	N1	443.99	885.12	429.33	586.15	1235.17	2012.96	925.34	1391.15
	N2	543.03	917.76	558.45	673.08	1439.72	2155.19	974.29	1523.07
	N3	612.70	1045.60	618.66	758.98	1745.61	2330.19	1021.74	1699.18
	Mean	462.84	775.03	464.52	567.46	1208.62	1728.08	833.83	1256.84
Mean	N0	328.77	328.77	328.77	328.77	598.83	598.83	598.83	598.83
	N1	549.80	988.43	484.84	674.36	1423.73	2094.13	1027.07	1514.98
	N2	646.29	1017.41	558.95	740.88	1588.12	2257.00	1201.81	1682.31
	N3	700.88	1102.27	619.99	807.71	1795.48	2425.75	1269.92	1830.38
	Mean	556.44	859.22	498.14		1351.54	1843.93	1024.41	
LSD 0.05		A: 2.65, B: 3.62, C:3.13 A*B: 5.12, A*C: 4.44, B*C:6.27 & A*B*C: 8.88				A: 7.14, B: 10.09, C: 8.74, A*B: 14.28, A*C: 12.36, B*C:17.49 & A*B*C: 24.73			

Data are displayed as mean (n = 3). Least Significant Difference (LSD) test at ($p \leq 0.05$) for factor A: growth seasons, factor B: N levels (control N0=no N addition, N1=60, N2=90 and N3=120 kg N fed^{-1}), factor C: N sources (Urea (U.), ammonium nitrate (A.N.) and ammonium sulphate (A.S.)) and the interaction effects: A*B, A*C, B*C & A*B*C.

Conclusions

Lettuce plants accumulated the highest contents of $N\text{-}NO_3$ after application of highest rates of nitrogen fertilizer in the form of ammonium nitrate in winter season. High rate of nitrogen application increase plant nitrate content without increasing the yield. Urea proved to be the optimum form, resulting in high yield of lettuce head, highly nutrients content and relatively modest nitrate accumulation. Also, the combination of higher light (summer season) and urea N-fertilizer at 90 Kg N fed^{-1} showed the highest positive impacts with highest lettuce yield and medium nitrate contents. Additional studies are required to investigate the optimal combination of light/nitrogen form and level for sustainable lettuce production of considering both yield and nutrition.

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