

Full Length Research Paper

Responses of lettuce to irrigation levels and nitrogen forms

S. Bozkurt^{1*}, G. S. Mansuroğlu¹, M. Kara¹ and S. Önder²

¹Department of Greenhouses, Samandag Higher Vocational Collage, Mustafa Kemal University, 31800 Samandag / Hatay, Turkey.

²Department of Agricultural Structure and Irrigation, Faculty of Agriculture, Mustafa Kemal University, 31034 Hatay, Turkey.

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This study was conducted to investigate the effects of different irrigation levels and nitrogen forms on yield, quality and water use efficiency of lettuce (*Lactuca sativa* var. *longifolia* cv. *Lital*) under greenhouse conditions during the periods November 2003 - February 2004 in the Eastern Mediterranean region of Turkey. Four irrigation treatments with a drip irrigation system were based on adjustment coefficients (Kcp) (0, 0.75, 1.0, and 1.25) of Class A pan evaporation. Nitrogen (N) treatments were consisted of ammonium nitrate (N_{AN}) and ammonium sulfate (N_{AS}) forms. As the yield and quality parameters of plant; mean marketable head weight, number of total and marketable leaves, plant height and diameters, root wet weight, plant dry weight, core diameters and tightness of head were determined. N forms significantly affected plant diameter and number of total and marketable leaves. Yield and other yield components were not affected by different N forms. Irrigation levels had significant ($p < 0.01$) different effects on yield and yield components except for plant dry weight. The results showed that the highest yield was obtained from N_{AN}xKcp₁₀₀ interaction plot. The water use efficiency (WUE) and the irrigation water use efficiency (IWUE) increased as the irrigation level reduced.

Key words: Drip irrigation, irrigation level, nitrogen, fertigation.

INTRODUCTION

Lettuce is the first cultivated salad crop and commercialized internationally (Abu-Rayyan et al., 2004). It is the most popular vegetable according to the highest consumption rate and economic importance throughout the world (Coelho et al., 2005). Water availability is generally the most important natural factor limiting the widespread and development of agriculture in arid and semi-arid regions. Turkey is characterized as semi-arid region and there are not irrigated large areas due to lack of irrigation water. Due to the number of benefits agriculture in greenhouse has also increased in recent years (Kadayıfçı et al., 2004). One challenge in greenhouse production is to design policies for cropping system management that improve product quality and control environmental impacts (Tourdonnet et al., 2001).

Many vegetable species are shallow-rooted and sensitive

to mild water stress. In lettuce, where the harvested part of the plant is the photosynthetic leaf area, it is especially important to maintain optimal growth through the application of water and nitrogen (Gallardo et al., 1996). Crop responses to different water applications have been used to determine irrigation strategies for optimal yield and maximum efficiency of water use for many crops.

N-form affected growth and yield of many vegetables (Gamiely et al., 1991). In plant nutrition, the main difference between nitrate (NO₃) and ammonium (NH₄) is that high rates of ammonium are highly toxic to plants since free ammonium irreversibly disrupts the structure of the thylakoid membrane (Wakiuchi et al., 1971; Simonne et al., 2001). Total N fertilizer recommendation for lettuce varies from 150 to 200 kg ha⁻¹, minus available mineral nitrogen in the root zone (Doerge et al., 1991; Sorensen et al., 1994; Karam et al., 2002). Hoque et al. (2008) reported that a number of factors influence NO₃ and NO₂. These include the type, amount and form of N fertilizer (Elia et al., 1998), as well accumulation in vegetables. as the geographical region and season of harvest as the geogra-

*Corresponding author. E-mail: sbozkurt33@gmail.com,
sbozkurt@mku.edu.tr Tel: +90.326.5128133. Fax:
+90.326.5130237.

Table 1. Some soil characteristics of experimental plots.

Soil depth (cm)	Texture	SP (%)	FC (g g ⁻¹)	WP (g g ⁻¹)	BD (g cm ⁻³)	ECe (dS m ⁻¹)	pH
0 - 30	Clay-Loam	50.97	40.96	17.07	1.66	2.8	7.8
30 - 60	Clay-Loam	50.33	44.50	18.96	1.56	3.1	7.2

SP: Saturation point, FC: Field capacity, WP: Wilting point, BD: Bulk density

Table 2. Some climatic data of the experimental area (inside and outside of the greenhouse).

Month	Temperature (°C)									Humidity (%)		
	Maximum			Minimum			Mean			In	Out	LT
	In	Out	*LT	In	Out	LT	In	Out	LT			
Nov.2003	26.0	20.8	30.0	13.4	11.4	0.0	18.2	15.7	15.6	80	73	71
Dec.2003	18.0	14.9	27.1	11.0	8.3	-1.1	13.9	11.4	11.4	84	77	73
Jan.2004	16.2	13.3	19.8	8.7	6.9	-2.2	12.1	9.9	9.7	82	78	74
Feb.2004	15.1	13.7	23.5	10.9	7.1	-1.0	13.3	10.8	10.4	83	75	73

* LT, Long term (1980 - 2001) in outdoors.

phical region and season of harvest (Walters, 1991). High nitrate levels, especially under adverse conditions such as drought, frost, unseasonable or prolonged cool temperatures, hail, shade and disease, high levels of soil nitrogen and soil mineral deficiencies or herbicide damage, can cause high nitrate accumulation (Safaa and Fattah, 2007).

The effects of different N forms with different irrigation levels on yield and yields components of lettuce are poorly examined under controlled environments. Therefore, the objectives of this study were to evaluate the effects of different nitrogen forms and irrigation levels on the yield and yield components of lettuce. Evapotranspiration (ET), irrigation water use efficiency (IWUE) and water use efficiency (WUE) were also investigated at the different irrigation levels.

MATERIALS AND METHODS

Lettuce (*Lactuca sativa* var. *longifolia* cv. *Lital*) was cultivated from November 2003 to February 2004 in a polyethylene covered greenhouse on the experimental station of the Samandag Higher Vocational Collage, University of Mustafa Kemal, located in Samandag, - Hatay, Turkey, with the latitude 36° 04' N, and the longitude 35° 57' E and 3.1 m above sea level.

Soil samples taken from 0 - 60 cm soil depth had 0.53% total organic matter, 21% CaCO₃, 0.18% total N, 12.35 mg kg⁻¹ total P₂O₅, 301.27 mg kg⁻¹ total K₂O, 9.15 mg kg⁻¹ Fe, 1.53 mg kg⁻¹ Zn, 6.4 mg kg⁻¹ Mn and 1.86 mg kg⁻¹ Cu. Some other properties of the soil in experimental plots are given in Table 1. Water table was also observed below 90 cm soil profile.

Samandag has a typical Mediterranean climate with hot-dry summers and mild-rainy winters. Climatic data of experimental area for the experimental periods are given in Table 2. The mean temperatures ranged between 12.1 and 18.2°C and the mean relative humidity changed from 80 to 84% in the greenhouse.

Irrigation water was obtained from a well in the study. The irrigation water sampled at the beginning of the study was analyzed

and classified by standard procedure of Anonymous (1954). According to the results, the water is C₃S₁ class and has no serious harmful effect on plant growing. Some other quality parameters determined in the laboratory were EC = 1.46 dS m⁻¹, pH = 7.91, TDS = 1180 mg L⁻¹, SAR = 2.03, Ca = 1.32, Mg = 7.39, Na = 4.24, K = 0.43, CO₃ = 0.75, HCO₃ = 7.00, Cl = 4.69 and SO₄ = 0.94 meq L⁻¹.

The experiment was designated as split plots with three replications. Main plots were the forms of N fertilizer and the sub-plots were irrigation levels derived from cumulative evaporation in a Class A pan between two irrigation events. In the experiment, four different irrigation levels and two nitrogen forms were tested as treatments. The irrigation levels were full irrigation (Kcp₁₀₀), 75% of full irrigation (Kcp₇₅; 25% deficit), 125% of full irrigation (Kcp₁₂₅; 25% excess) and no irrigation (Kcp₀) treatments. The nitrogen treatments were ammonium nitrate (N_{AN}), ammonium sulfate (N_{AS}) forms of N fertilizers and no-nitrogen (N₀).

The plots were 26.1 m in length and 1.2 m in width and 1.0 m apart. Each plot had four plant rows. The plants were transplanted in the greenhouse at the five to six true-leaf stages at a spacing of 0.3 x 0.3 m on November 18, 2003. The mean marketable head weight (yield), number of total and marketable leaves, plant height, head diameter, core diameter, plant dry weight and tightness of head were determined just before and after the harvest by using standard procedures.

A common recommended fertilization program was followed in the experimental plots. All plots received the same amounts of fertilizer consisted of 100 kg ha⁻¹ P₂O₅ and 200 kg ha⁻¹ K₂O and 150 kg ha⁻¹ N. All fertilizers were applied with drip irrigation in three split application. Two sources of N were used namely: ammonium sulfate (AS, 21% N) and ammonium nitrate (AN, 33.5% N). A constant rate of both P-fertilizer (Mono potassium phosphate 52% P₂O₅, 34% K₂O) and K-fertilizer (potassium sulfate 51% K₂O) were added to all treatment plots.

The surface drip lateral lines served one drip line for two crop rows were installed after planting. The drip irrigation laterals were 16 mm in diameter. The drippers were inline type and 0.20 m apart from each other and had 2.75 L h⁻¹ flow rate at 100 kPa pressure. The irrigation system has a typically control unit consisted of a pump, fertilizer tank, gravel and disc filters, control valves, pressure gauges and a flow meter.

After stand establishment on 20 November, 2003; first irrigation

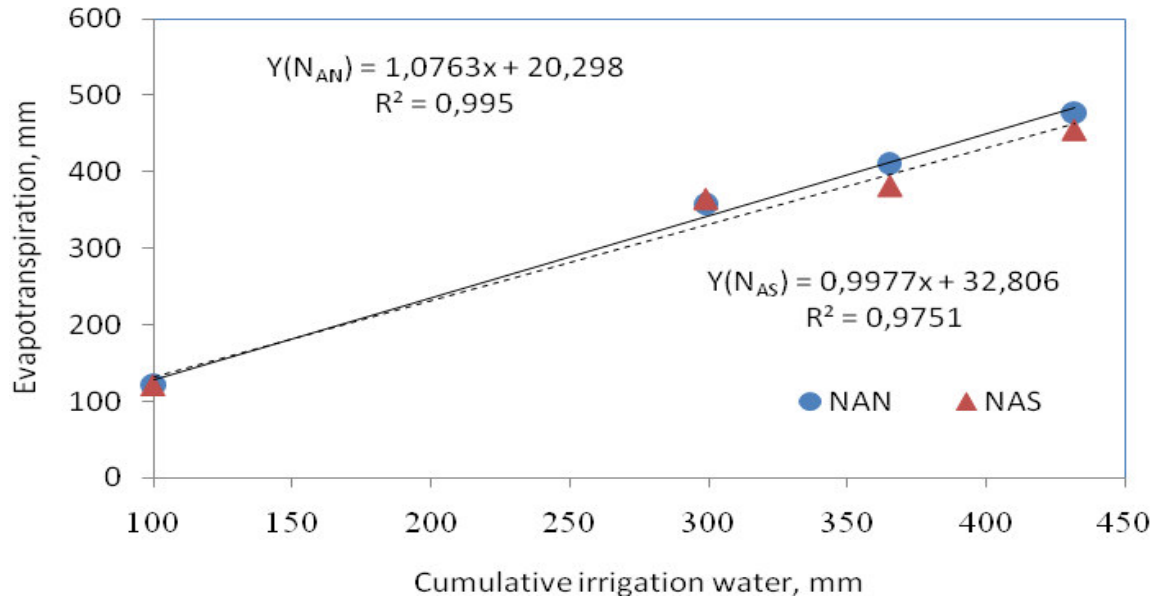


Figure 1. Relationship between irrigation water and evapotranspiration in N treatments.

(100 mm) was applied to bring the soil water content up to the level of field capacity in 0 – 60 cm soil depth to all treatments. Irrigations were started when the readings of tensiometer placed in 30 cm soil depth on the full irrigation (K_{cp100}) plot approached 25 kPa.

The amount of irrigation water was calculated by using following Eq. (1):

$$I = A \cdot E_{pan} \cdot K_{cp} \quad (1)$$

Where; I is the amount of irrigation water (L), A is the plot area (m^2), E_{pan} is the amount of cumulative evaporation during an irrigation interval (mm), K_{cp} is the crop-pan coefficient. Class A pan was located at the center of the experimental plots in the greenhouse. Daily readings of the Class A pan evaporation was made in the mornings during the study.

Soil water contents were measured gravimetrically at 30 cm increments down to 90 cm during the study. Soil water status of each irrigation plots were also monitored by using tensiometers set at 15 and 30 cm below the surface of the beds, mid-way between rows of plants. Evapotranspiration (ET) for different watering regimes was calculated by using the soil water balance equation (James, 1988):

$$ET = I + P \pm \Delta SW - D_p - R_f \quad (2)$$

Where; ET is the seasonal evapotranspiration (mm), I is the irrigation water (mm), P is the precipitation (mm), ΔSW is the change in the soil water storage (mm) in 60 cm soil profile, D_p is the deep percolation (mm) and R_f is the amount of runoff (mm). Since drip irrigation was used in the greenhouse, runoff and precipitation were assumed to zero. The amount of irrigation water applied throughout the study was controlled, thus, deep percolation was also zero.

Water use efficiency (WUE) and irrigation water use efficiency (IWUE) were calculated as marketable lettuce weight (yield) divided by seasonal evapotranspiration and seasonal irrigation water applied, respectively (Howell et al., 1990). Data were subjected to statistical analysis using the MSTATC software (Michigan State University) and the treatment means were compared by LSD (Least Significant Differences) test at $p < 0.05$ significant level.

RESULTS AND DISCUSSION

Irrigation water and Evapotranspiration (ET)

In the study, amounts of irrigation water were calculated with pre-determined coefficients (0, 0.75, 1.0 and 1.25) of Class A pan evaporation as the irrigation treatments. Thus, plots were irrigated with different amounts of water. The first irrigation was applied on 20 November, 2003 and last irrigation was made on 24 January, 2004. Five irrigation applications were performed in all experimental plots except for non-irrigation plots (K_{cp0}) according to soil water deficit. Seasonal irrigation amounts were 298.9, 365.2, and 431.5 mm in treatments of K_{cp75} , K_{cp100} and K_{cp125} , respectively. In control plots (K_{cp0}), irrigation water (100 mm) was performed only once after transplanting of crops for subsistence of crops. Among all treatments in the experiments, the maximum ET (477 mm) was obtained under $N_{AN}K_{cp125}$ treatment while minimum ET (122.0 mm) was determined under K_{cp0} treatment. The lowest water consumption at K_{cp0} treatments could be related to lack of soil moisture resulting from no irrigation. Increasing the amount of irrigation water increased the water availability for evapotranspiration. In order to clarify the effects of irrigation on ET, regression analysis was performed. There was a significant linear relationship ($R^2 = 0.995$ in N_{AN} and $R^2 = 0.975$ in N_{AS} treatments) between the irrigation water applied and the crop evapotranspiration as shown in Figure 1.

Rates of ET were essentially low at the early stages of vegetative growth (from 0 to 20 Days After Transplanting, DAT), then increased gradually (from 21 to 68 DAT) by the end of the growing season, when crops had reached

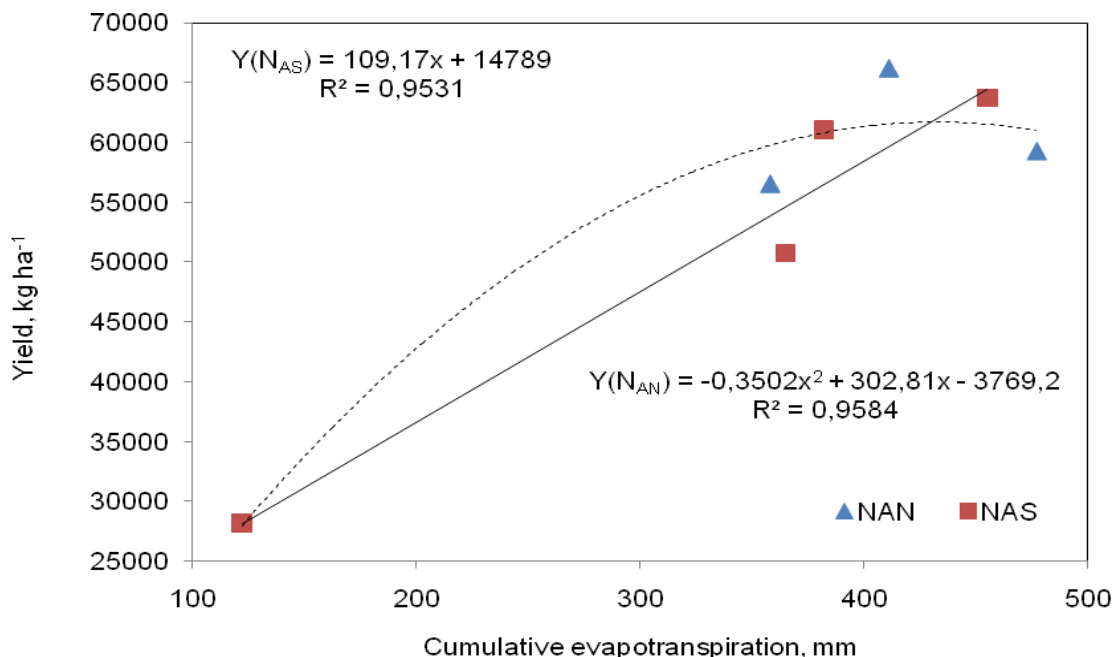


Figure 2. The relationship between lettuce yield and cumulative evapotranspiration under different irrigation levels in ammonium nitrate (N_{AN}) and ammonium sulfate (N_{AS}) treatments.

Table 3. Water use efficiency (WUE) and irrigation water use efficiency (IWUE) of lettuce under four different crop–pan coefficients (Kcp).

Treatments	Yield, kg ha ⁻¹	Irrigation water, mm	Evapotranspiration, mm	IWUE, kg m ⁻³	WUE, kg m ⁻³	
N_{AN}	Kcp ₀	28158.0	100.0	122.0	28.2	23.1
	Kcp ₇₅	56571.3	298.9	358.0	18.9	15.8
	Kcp ₁₀₀	66226.7	365.2	411.0	18.1	16.1
	Kcp ₁₂₅	59296.1	431.5	477.0	13.7	12.4
N_{AS}	Kcp ₀	28158.0	100.0	122.0	28.2	23.1
	Kcp ₇₅	50732.5	298.9	365.0	17.0	13.9
	Kcp ₁₀₀	61082.4	365.2	385.0	16.7	15.9
	Kcp ₁₂₅	63730.8	431.5	455.0	14.8	14.0

had reached the maximum number of mature leaves. Likewise, Karam et al. (2002) demonstrated similar results in their former study.

Lettuce showed different yield responses to the nitrogen forms in each irrigation levels. While there was second degree polynomial relationship between the crop evapotranspiration and the yield of lettuce in N_{AN} form, linear relationship was found in N_{AS} treatment (Figure 2). Yield increased as the water application increased up to 66226.7 kg ha⁻¹ for lettuce with an associated seasonal evapotranspiration of 411 mm in $N_{AN}Kpc_{100}$ treatment. Similar increases were observed in $N_{AS}Kcp_{125}$ treatments where increase of yield was up to 63730.8 kg ha⁻¹ with an associated seasonal evapotranspiration of 455 mm. Coelho et al. (2005) demonstrated similar results that the

total maximum and marketable yields were 6985 and 5931 g m⁻² respectively in 100% pan evaporation replacement (Kcp_{100}).

WUE were ranged from 12.4 kg m⁻³ of water in Kcp_{125} to 23.1 kg m⁻³ of water in Kcp_0 treatments. IWUE were ranged from 13.7 kg m⁻³ of water in Kcp_{125} to 28.2 kg m⁻³ of water in Kcp_0 treatments (Table 3). These results are in agreement with the results of Sammis et al. (1988). Karam et al. (2002) reported that the water deficit treatments had lower WUE than full irrigation. In contrast to this, we determined that the WUE and IWUE increased by the reduction of irrigation. Similarly, Gallardo et al. (1996) reported that the lettuce dry matter and fresh weight were linearly related to the total water use, leading to similar water use efficiency values for all irrigation

treatments.

Yield and yield components

Yield responses of lettuce to four different irrigation levels and two N forms plus N_0 were determined in the experiment. Analyses of variance were performed to determine the effects of N forms and irrigation levels on lettuce yield and yield components (Table 4). N forms had significant ($p < 0.05$) effects on diameter of plants and number of total and marketable leaves. Yield and other yield components were not affected by N forms. Irrigation levels had significant ($p < 0.01$) effects on yield and yield components except for plant dry weight. Interaction effects of N forms and irrigation levels had significantly different effects on number of total and marketable leaves at $p < 0.05$ level.

Treatment means were separated by applying LSD ($p < 0.05$) test (Table 5). Significant differences were observed among the treatments with regard to the yield, plant diameters, core diameters and number of total and marketable leaves. The highest yield was obtained from the treatment of N_{AN} , while the lowest yield was observed in N_0 treatment. Although, plant dry weight was not found different between N_{AS} and N_{AN} treatments, the N_{AS} promotes core diameter, root wet weight and tightness of heads more than N_{AN} . Abu-Rayyan et al. (2004) reported that the ammonium sulfate proved to be the optimum N form, leading to high dry matter content in lettuces. It is known from the some literatures (Tusun and Ustun, 2004; Safaa and Fattah, 2007) that nitrogen form as ammonium application reduces plant nitrate content. In view of this, $N_{AS}Kcp_{125}$ treatment could result in only minor reduction of yield, although the highest yield was found in $N_{AN}Kcp_{100}$ treatment (Table 6).

Irrigation treatments had significant ($p < 0.05$) different effects on all examined parameters except for plant dry weight (Table 4). LSD grouping of yield and yield components from the irrigation treatments indicated that Kcp_0 was in the last group (Table 5). The highest yield, plant and core diameters, head tightness and number of marketable leaves were obtained from Kcp_{125} , while the lowest values were observed in Kcp_0 (control). Acar et al. (2008) reported that different irrigation levels did not significantly affect mean leaf number, head height and head circle. This is in contrast with our results except for head circle (plant diameter).

Although, irrigation levels had significant effects on plant and root wet weight, they did not affect the plant dry weight. Similarly, Gallordo et al. (1996) reported that the decrease in water applied from field capacity (FC) to 87% of FC generally did not affect final dry matter but slightly decreased the fresh weight. They also declared that the mean dry matter production and plant fresh weight for the 45% of FC treatment for the three cultivars in relation to the FC treatment were 72 and 58%, respectively, indica-

ting that the decreased water supply had a greater effect on the fresh weight than on the dry matter.

Additionally, Soundy et al. (2005) reported that the root dry weights were unaffected by moisture deficit however shoot dry weight and leaf N content increased with increasing moisture deficit in the media. Since, the interaction effects of the treatments on the yield and the yield components could differ from their individual effects, LSD classifications were carried out using N x Kcp interaction. LSD grouping of number of total and marketable leaves from the N x Kcp interaction indicated that $N_{AN}Kcp_{100}$ was in the first group and the Kcp_0 of N_0 , N_{AN} and N_{AS} (control) was in the last group.

Although interaction effects of N and Kcp levels on yields was not statistically significant, the highest yield ($595.7 \text{ g plant}^{-1}$) was obtained from Kcp_{100} irrigation level in N_{AN} treatment. In $N_{AN}Kcp_{125}$ irrigation plots, yield decreased by 10.5% (533 g plant^{-1}) according to yields of $N_{AN}Kcp_{100}$ plots. This reduction might be resulted from N losses especially deep percolation, volatilization and denitrification process. It might also be caused by the better water usage and better soil-water-air combination with higher aeration of the root zone in Kcp_{100} plots. In the 25% water deficit (Kcp_{75}) with N_{AN} fertigation, yield decreased with reduction of 14.5% from $595.7 \text{ g plant}^{-1}$ to 509 g plant^{-1} . Karam et al. (2002) reported that water deficit produced significant differences in fresh weight of individual heads ($p < 0.05$).

The average fresh weight of the well-irrigated plants (I - 100 indicated to receive 100% of the soil water depletion) in their report was 757 g, whereas I - 80 and I - 60 treatments resulted in 14 and 39% reduction in fresh weight, respectively. Additionally, Acar et al. (2008) also declared that the head weights were 355.17, 340.3 and 338.43 g from S1 (receiving 100% of the soil water depletion), S2 (80%) and S3 (60%) water applications, respectively. Results of our study were in good agreement with the findings reported by Karam et al. (2002). Hence, Kcp_{100} treatment appears to be more practical than the Kcp_{125} for the sustainable use of water resources.

Conclusion

The results of the current study indicated that different irrigation levels had significant effects on the majority of lettuce yield components at Kcp_{100} and Kcp_{125} levels. Since there were only minor differences between yields of lettuce obtained from Kcp_{100} and Kcp_{125} irrigation levels, Kcp_{100} emerges as a more suitable practice and might be recommended to tolerate the negative effects of excess water application to the ecology and for a better water economy especially in arid regions of the world.

N forms significantly affected the plants diameter and the number of total and marketable leaves. Yield and other yield components were not affected by N forms. Hence, ammonium sulfate fertilizer should be more be-

Table 4. Variance analyses of yield and yield components (F-Values).

Source of variance	df	Yield, g plant ⁻¹	Plant height, cm	Plant diameter, cm	Core diameter, mm	Root wet weight, g	Head tightness (1 - 5)	No of Total leaf, no plant ⁻¹	No of marke- table leaf, no plant ⁻¹	Plant dry weight, g
Form of N	2	4.59	2.09	14.87*	6.79	1.93	1.83	8.37*	7.96*	2.17
Kcp	3	64.89**	24.40**	42.05**	39.31**	8.31**	15.77**	27.54**	37.60**	9.82
NxKcp	6	2.36	1.11	1.81	2.17	1.68	1.01	3.19*	3.20*	1.30
CV (%)		10.99	4.16	9.20	11.68	15.3	21.8	7.65	8.43	28.75

*significant at $p < 0.05$, **significant at $p < 0.01$, df: Degrees of freedom, CV: Coefficient of variation.

Table 5. The LSD test results of yield and yield components under different nitrogen form and irrigation levels[†].

Treatments	Yield, g plant ⁻¹	Plant height, cm	Plant diameter, cm	Core diameter, mm	Root wet weight, g	Head tightness (1 - 5)	No of Total leaf, no plant ⁻¹	No of marketable leaf, no plant ⁻¹	Plant dry weight, g
N ₀	382.6	36.8	36.5b	89.3	10.6	3.0	36.6b	32.0b	6.8
N _{AN}	472.7	38.3	43.4a	103.9	10.4	3.5	41.3a	37.3a	8.3
N _{AS}	457.8	37.8	41.0a	113.5	12.4	3.8	40.0a	36.3a	8.3
LSD _{0.05}	NS ^{††}	NS	3.6	NS	NS	NS	3.3	3.9	NS
Kcp ₀	253.0c	34.0c	28.7c	66.3c	8.7b	2.0b	31.7b	26.3c	4.3
Kcp ₇₅	444.4b	37.6b	41.8b	104.3b	11.9a	3.5a	40.0a	36.2b	8.4
Kcp ₁₀₀	523.6a	39.8a	44.2ab	118.3a	12.3a	4.1a	42.8a	38.9ab	9.4
Kcp ₁₂₅	529.8a	39.1ab	46.7a	120.1a	11.7a	4.1a	42.8a	39.3a	8.9
LSD _{0.05}	47.6	1.6	3.7	11.8	1.7	0.7	3.0	2.9	NS

[†]Means followed by the same letter(s) in the same column are not significantly different at 0.05 level, ^{††}NS: non significant.

neficial for the environmental practices. However, further research is required to assess the relative effect of fertilizer forms and amounts under detailed irrigation levels.

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Table 6. Interaction effects of different nitrogen form and irrigation levels on yield components[†].

Treatments		Yield, g plant ⁻¹	Plant height, cm	Plant diameter, cm	Core diameter, mm	Root wet weight, g	Head tightness (1-5)	No of Total leaf, no plant ⁻¹	No of marke- table leaf, no plant ⁻¹	Plant dry weight, g
N ₀	Kcp ₀	253.0	34.0	28.7	66.3	8.7	2.0	31.7f	26.3f	4.3
	Kcp ₇₅	368.2	36.4	35.7	86.8	10.3	2.8	36.0ef	31.3ef	6.6
	Kcp ₁₀₀	425.8	37.7	39.2	97.0	11.2	3.3	38.3de	34.0de	7.7
	Kcp ₁₂₅	483.4	38.9	42.7	107.2	12.1	3.9	40.3cde	36.3cde	8.8
N _{AN}	Kcp ₀	253.0	34.0	28.7	66.3	8.7	2.0	31.7f	26.3f	4.3
	Kcp ₇₅	509.0	38.0	46.0	105.3	12.3	4.0	44.3abc	40.7abc	8.3
	Kcp ₁₀₀	595.7	41.0	50.3	129.7	10.7	4.3	48.0a	44.0a	12.0
	Kcp ₁₂₅	533.0	40.0	48.7	114.3	10.0	3.7	41.3cd	38.3bcd	8.3
N _{AS}	Kcp ₀	253.0	34.0	28.7	66.3	8.7	2.0	31.7f	26.3f	4.3
	Kcp ₇₅	456.0	38.3	43.7	120.7	13.0	3.7	39.7cde	36.7cd	10.3
	Kcp ₁₀₀	549.3	40.7	43.0	128.3	15.0	4.7	42.0bcd	38.7bcd	8.7
	Kcp ₁₂₅	573.0	38.3	48.7	138.7	13.0	4.7	46.7ab	43.3ab	9.7
LSD _{0.05}		**NS	NS	NS	NS	NS	NS	5.2	5.1	NS

[†]Means followed by the same letter(s) in the same column are not significantly different at 0.05 levels; **NS: non significant.

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