

The impact of different growth media and ammonium-nitrate ratio on yield and nitrate accumulation in lettuce (*Lactuca sativa* var. *longifolia*)

Selçuk SÖYLEMEZ*

Harran University, Faculty of Agriculture, Department of Horticulture, Sanliurfa, Turkey; sssoylemez@harran.edu.tr

Abstract

Ammonium (NH_4^+) to nitrate (NO_3^-) ratio and growth media significantly alter plant development and NO_3^- accumulation in lettuce. Nitrate accumulation is regarded harmful for environment and human health. The quality of lettuce is assessed by NO_3^- concentration, size, and weight. This study determined the impact of five different growth media (cocopeat, peat, bark, perlite and rockwool) and four different $\text{NH}_4^+:\text{NO}_3^-$ ratios (0:100, 20:80, 40:60 and 60:40) on NO_3^- accumulation in lettuce, yield, and several growth attributes. The experimentation was conducted according to open feeding system of soilless agriculture. The 'Cosmos' variety of lettuce was used as experimental material in the study. Growth mediums and $\text{NH}_4^+:\text{NO}_3^-$ ratios significantly altered NO_3^- accumulation, head, leaf, stem and root traits. The results revealed that instead of growing lettuce with NO_3^- only in peat and rockwool, addition of NH_4^+ (20:80 of $\text{NH}_4^+:\text{NO}_3^-$) into nutrient solution increased head weight. While head weight increased in perlite medium with the addition of NH_4^+ , it decreased in cocopeat and bark media. It is concluded that growth media and $\text{NH}_4^+:\text{NO}_3^-$ ratios pose significant impacts on NO_3^- accumulation in leaf and that the increase in NH_4^+ ratio decreased NO_3^- accumulation in all growing media. Therefore, it is recommended that NH_4^+ should be added in the nutrient solution to decrease NO_3^- accumulation, which will ultimately improve yield and quality of lettuce.

Keywords: NH_4^+ ; NO_3^- ; plant nutrition; soilless agriculture; substrate

Introduction

Proper fertilization and provision of suitable ecological conditions is necessary to get higher yield best from crop plants. Nevertheless, production sustainability and safety of environment and human health must be considered while establishing the fertilization programs for getting higher yield and quality. Several other factors, including climate, developmental stage of plant, soil structure and nature of plants should also be considered while devising fertilization programs. It is commonly observed that high fertilizer input increases yield; however, leads to low quality and several other problems. Significant nitrate (NO_3^-) accumulation, which contains high amount of nitrogen (N) is observed in below and aboveground parts of plants due fertilizers (Güneş, 1994; Ayaz and Yurttagül, 2006; Zandvakili *et al.*, 2019). It is reported that the use of fertilizers containing high amount of NO_3^- and nitrite (NO_2^-) negatively affects human health and causes anemia. These also form carcinogenic nitrosamines by reacting with the secondary amines in human body (Oruç and Ceylan, 2001; Ayaz and Yurttagül, 2006; Özdeştan and Üren, 2010). Nitrate and NO_2^- are indigenous to soil, surface

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and ground water, air, and plants (Aköz, 1981). Nitrate turns into NO_2^- and become harmful since the later decrease's oxygen-carrying capacity of blood when reacts with hemoglobin and turns Fe^{+2} into Fe^{+3} . This situation known as Blue Baby Syndrome is very dangerous for children (Oruç and Ceylan, 2001; Özdestand and Üren, 2010). It is reported that acute toxicity of NO_2^- is frequent and leads to death, whereas acute toxicity of NO_3^- is rare (Özdestand and Üren, 2010). Approximately 87% of NO_3^- intake to human body comes through vegetable consumption (Özdestand and Üren, 2010). It is suggested that NO_3^- is a natural component of both vegetables and fruits and used as a preservative substance in meat and fish products (Ayaz and Yurttagül, 2006).

The NO_3^- accumulation in plant tissues increases depending on nitrogenous fertilizers, low activity of nitrate reductase, low pH values, low light and soil moisture, high temperature, cloudy weather, and drought etc. (Rao and Rains, 1976; Ayaz and Yurttagül, 2006; Yardım, 2009; Çokuysal *et al.*, 2016). The velocity of biochemical processes is slow and NO_3^- level is high under low light availability. Moreover, cultivation season, storage and geographical conditions also affect NO_3^- accumulation. Nitrate content in plants is related to their biological activities (Stepowska and Kowalczyk, 2001). Nitrate is digested in plant tissues by nitrate reductase enzyme. Nitrate accumulation increases when the activity of this enzyme is inhibited. Molybdenum (Mo) has a key role in the synthesis of nitrate reductase, and it is cofactor required for the enzyme (Ayaz and Yurttagül, 2006). The deficiency of Mo in growth medium inhibits the activity of nitrate reductase; thus, NO_3^- accumulation may increase. Molybdenum deficiency leads to high NO_3^- accumulation in many plant species (Moncada *et al.*, 2018). It is suggested that NO_3^- content in lettuce can be decreased through addition of Mo to the medium (Moncada *et al.*, 2018).

Zhao *et al.* (2016) reported that N has significant effect on plant growth, nutrient intake, and physiological processes of plants. Plants principally uptake N from soil in the form of NO_3^- or NH_4^+ , and their ratio can easily change depending on pH of root zone, age and type of plant, quantity of light, and presence/absence of other ions. While NO_3^- generally taken in acidic conditions, NH_4^+ is taken under alkaline conditions (Yardım, 2009; Çokuysal *et al.*, 2016). The whole amount of N required by plants in soilless agriculture system can be given in NO_3^- form. However, it is suggested to keep 15% NH_4^+ in nutrient solution due its positive effects (Gül, 2012).

Growing vegetable in soilless agriculture is steadily around the world. Several growth media made up of organic or inorganic materials are used in soilless agriculture. Limited literature is available on the impact of growth media on NO_3^- accumulation in plants. Furthermore, there are limited studies conducted on the impact of $\text{NH}_4^+:\text{NO}_3^-$ ratio on plant growth and NO_3^- accumulation in lettuce. Therefore, this study assessed the impacts of various growth media and different $\text{NH}_4^+:\text{NO}_3^-$ ratios in nutrient solution on yield, plant growth and NO_3^- accumulation in lettuce. This study aims to determine the appropriate $\text{NH}_4^+/\text{NO}_3^-$ ratios according to the growing medium and thus to improve the quality of the crop by reducing nitrate accumulation in lettuce.

Materials and Methods

Experimental site

This study was conducted in polycarbonate greenhouse located at Faculty of Agriculture, Harran University, Şanlıurfa, Turkey during winter growing season of 2019.

Treatments

The 'Cosmos' lettuce (*Lactuca sativa* var. *longifolia*) variety was used as experimental material in the study. Many organic and inorganic media are used in the production of vegetables in greenhouses. In the study, 5 different organic and inorganic origin media, which are mostly used in production, such as cocopeat, perlite, peat, bark and rockwool were preferred. Four different $\text{NH}_4^+:\text{NO}_3^-$ ratios each containing 150 ppm N, were applied to all growing media. The amount of N and other elements in solution was kept stable. The $\text{NH}_4^+:\text{NO}_3^-$ ratios were adjusted by changing the amounts of ammonium sulphate, calcium sulphate and calcium nitrate.

The EC of nutrient solution was set at 1.8 dS m⁻¹ and pH value was 5.8. The NH₄⁺:NO₃⁻ ratios were i) 0% NH₄⁺-100% NO₃⁻ (0 ppm NH₄⁺ and 150 ppm NO₃⁻), ii) 20% NH₄⁺-80% NO₃⁻ (30 ppm NH₄⁺ and 120 ppm NO₃⁻), iii) 40% NH₄⁺-60% NO₃⁻ (60 ppm NH₄⁺ and 90 ppm NO₃⁻) and iv) 60% NH₄⁺-40% NO₃⁻ (90 ppm NH₄⁺ and 60 ppm NO₃⁻).

The lettuce seedlings used in the experiments were bought from a local seedling firm. The seedlings were planted in 8-litre pots containing growth media on 1st December 2018 and harvested on 20th March 2019. All pots received same amount of fertilizer; however, different NH₄⁺:NO₃⁻ ratios were applied 10 days after planting. The formulation of nutrient solution proposed by Gül (2012) for lettuce growing in winter months was used in the current study (Table 1).

Table 1. Nutrient solution content used in the experimentation (mg L⁻¹)

N	P	K	Ca	Mg	Fe	Mn	Zn	B	Cu	Mo
150	50	150	150	50	5	0.5	0.05	0.5	0.03	0.02

Nitrate analysis

Nitrate contents in leaves were determined via salicylic acid method designed by Cataldo *et al.* (1975). 0.1 ml of the extracted leaf sample and 0.4 mL of 5% salicylic acid solution (dissolved in concentrated H₂SO₄) were mixed well. After 20 minutes at room temperature, 9.5 mL of 2N NaOH were added slowly to raise the pH above 12. Samples were cooled to room temperature and absorbance at 410 nm was determined in a spectrophotometer.

Head traits

The lettuces were harvested by cutting to growth media level. Afterwards, head diameter and length were measured by ruler. The heads were weighed in a precision scale for head fresh weight.

Leaf traits

The leaves in the harvested lettuce were separated from the stem and counted. The number of inner and outer leaves were counted separately. Total number of leaves were computed by adding inner and outer leaves. The leaves were weighed in a precision scale for fresh weight. Then, the leaves samples were dried in an oven at 65 °C until constant weight, and dry weight of leaves was recorded. Dry matter ratio was calculated by the equation 1 given below.

$$\text{Dry matter ratio (\%)} = \text{leaf dry weight} / \text{leaf fresh weight} \times 100 \quad (1)$$

Stem and root traits

Leaves in the plants harvested were separated from stem and stem were weighed in a precision scale for fresh weight. The stems were dried in an oven at 65 °C until constant weight, and dry weight of stem was recorded. Roots were removed from growth media, washed and their length was measured. Afterwards roots were dried according to the above procedure and then root dry weight was recorded. However, roots of the plants grown in rockwool medium were not evaluated since they were not completely cleaned.

Statistical analysis

The experiment was laid out according to factorial design where growth media were main factor, while NH₄⁺:NO₃⁻ ratios were regarded as sub-factor. All treatments had three replications. The collected data regarding plant attributes and NO₃⁻ contents were tested for normality first through Shapiro-Wilk normality test. The normality test revealed that data had normal distribution; therefore, original data were used in the statistical analysis. Two-way Analysis of variance (ANOVA) was used to test the significance in the data. Least

significant difference test at 5% probability was used to determine the difference among treatment means. All statistical computations were done on MSTAT-C statistics software.

Results

Nitrate content and head traits

Different growth media, $\text{NH}_4^+:\text{NO}_3^-$ ratios, and the interaction significantly altered NO_3^- accumulation (Table 2). The highest NO_3^- content was found in peat media, while the lowest was observed in perlite. Decreasing $\text{NH}_4^+:\text{NO}_3^-$ ratio increased NO_3^- accumulation. The highest NO_3^- accumulation was recorded for 0:100 $\text{NH}_4^+:\text{NO}_3^-$ ratio. However, the lowest NO_3^- accumulation was noted for 60% ammonium and 40% nitrate. As to interaction between medium and $\text{NH}_4^+:\text{NO}_3^-$ ratio, the highest nitrate accumulation was observed in cocopeat and peat media, in which the $\text{NH}_4^+:\text{NO}_3^-$ ratio was 0:100. The lowest nitrate accumulation was seen in perlite with 60:40 or 40:60 $\text{NH}_4^+:\text{NO}_3^-$ ratios in cocopeat, peat, bark and rockwool growth media.

Head weight was significantly influenced by growth media and interaction among $\text{NH}_4^+:\text{NO}_3^-$ ratio and growth media; however, $\text{NH}_4^+:\text{NO}_3^-$ remained non-significant. The highest head weight was noted for peat and cocopeat media, while rockwool resulted in the lowest head weight. Regarding interaction, the highest head weight was recorded for peat with 20:80 $\text{NH}_4^+:\text{NO}_3^-$ ratio. The lowest head weights were obtained from all nitrate ratios of rockwool.

Table 2. Various $\text{NH}_4^+:\text{NO}_3^-$ rates and the impacts of growth medium on head weight, head diameter and head length

Growth media		Nitrate content (mg kg^{-1})	Head weight (g)	Head diameter (cm)	Head length (cm)
Substrates	Cocopeat	1826.42 ± 271.07 ab	567.92 ± 37.63 a	19.04 ± 0.84 ab	28.88 ± 2.92 b
	Peat	1905.42 ± 239.05 a	583.42 ± 20.67 a	20.13 ± 1.33 a	31.75 ± 2.93 a
	Bark	1599.92 ± 215.19 bc	396.92 ± 13.82 c	17.50 ± 0.93 bc	30.58 ± 2.35 a
	Perlite	1524.33 ± 202.96 c	501.75 ± 17.02 b	19.21 ± 1.10 a	28.83 ± 1.75 b
	Rock wool	1568.33 ± 170.64 bc	316.58 ± 8.70 d	16.83 ± 0.24 c	24.08 ± 1.62 c
LSD		*299.2	**39.930	**1.685	**1.605
$\text{NH}_4^+:\text{NO}_3^-$	0:100	2521.07 ± 137.66 a	480.47 ± 30.07 a	17.13 ± 0.51 c	30.23 ± 0.87 a
	20:80	2056.80 ± 120.76 b	483.67 ± 30.91 a	17.20 ± 0.24 c	29.20 ± 1.01 ab
	40:60	1276.73 ± 57.65 c	472.53 ± 28.74 a	20.90 ± 1.02 a	27.40 ± 0.60 c
	60:40	884.93 ± 38.86 d	456.60 ± 32.63 a	18.93 ± 0.95 b	28.47 ± 0.97 bc
LSD		**358.1	NS	**1.507	**1.435

Different letters indicate significant differences at $P < 0.05$ by LSD test, * and ** represent $P < 0.05$ and $P < 0.01$, respectively. NS: Not significant. Data are shown as the mean values ± SE (for substrates $n=12$ and for nitrate ratios $n=15$).

Likewise, the impact of growing media, $\text{NH}_4^+:\text{NO}_3^-$ ratios and their interaction on head diameter was significant. The plants grown in peat and perlite media recorded the largest head diameter. However, the lowest head diameter was recorded for the plants grown on rockwool. The 40:60 $\text{NH}_4^+:\text{NO}_3^-$ ratio recorded the highest head diameter, while 0:100 and 20:80 ratios resulted in the lowest head diameter. Regarding interaction, the largest head diameter was noted for peat with 40:60 $\text{NH}_4^+:\text{NO}_3^-$ ratio. The lowest head diameter was recorded for bark medium with 60:40 $\text{NH}_4^+:\text{NO}_3^-$ ratio.

Head length was significantly altered by growth media, $\text{NH}_4^+:\text{NO}_3^-$ ratio and their interaction. The plants grown in peat and bark media had the highest head length whereas rockwool had the lowest head length values. Peat medium with 20:80 $\text{NH}_4^+:\text{NO}_3^-$ recorded the highest head length, whereas rockwool with 60:40, 40:60 and 20:80 $\text{NH}_4^+:\text{NO}_3^-$ ratios resulted in the lowest head length.

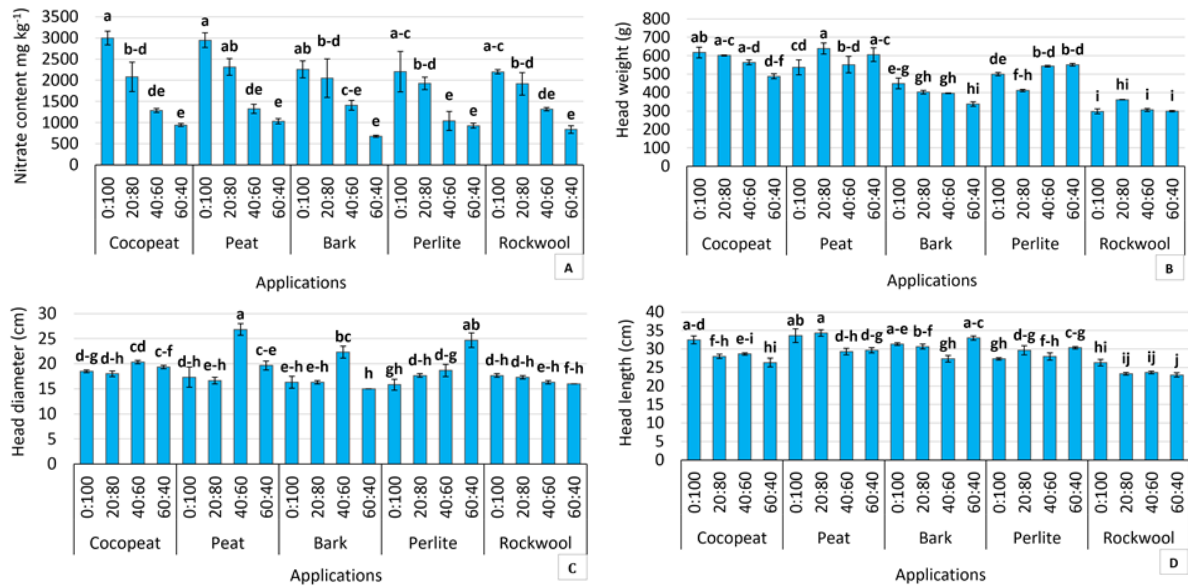


Figure 1. Interactive effect of growth media and $\text{NH}_4^+:\text{NO}_3^-$ ratios on the (A) nitrate, (B) head weight, (C) head diameter and (D) head length. Data are shown as the mean values \pm SE ($n=3$). Different letters above the bars indicate significant differences between the treatment ($P < 0.05$, LSD test).

Leaf traits

The values of inner, outer, and total leaf count, leaf fresh and dry weights, and dry weight percentage are given in Table 3 and Table 4. Growth media, $\text{NH}_4^+:\text{NO}_3^-$ ratios, and their interaction significantly affected outer leaf count. The highest outer leaf count was noted for rockwool. The highest and the lowest outer leaf count was noted for 60:40 and 20:80 $\text{NH}_4^+:\text{NO}_3^-$ ratios, respectively. Regarding interaction, cocopeat with 60:40 $\text{NH}_4^+:\text{NO}_3^-$ ratio recorded the highest outer leaf count (Figure 2A).

The $\text{NH}_4^+:\text{NO}_3^-$ ratios, growth media and interaction between growth media and $\text{NH}_4^+:\text{NO}_3^-$ ratio had significant effect on inner leaf count. The highest and the lowest inner leaf count was noted for cocopeat and rockwool, respectively. Generally, decreasing NO_3^- and increasing NH_4^+ ratio in nutrient solution improved inner leaf count. The highest and the lowest inner leaf count was recorded for 60:40 and 0:100 $\text{NH}_4^+:\text{NO}_3^-$ ratios, respectively. Regarding interaction, the highest and the lowest values for inner leaf count were recorded for cocopeat with 40:60 and rockwool with 40:60 $\text{NH}_4^+:\text{NO}_3^-$ ratio, respectively (Figure 2B).

Total leaf count was significantly affected by growth media and $\text{NH}_4^+:\text{NO}_3^-$ ratios; however, interactive effect of growth media and $\text{NH}_4^+:\text{NO}_3^-$ ratios was non-significant. Generally, decreased NO_3^- in solution increased total leaf count. The highest total leaf count was recorded for 60:40 $\text{NH}_4^+:\text{NO}_3^-$ ratio, 0:100 and 20:80 ratios resulted in the lowest total leaf count (Figure 2C).

Table 3. Various $\text{NH}_4^+:\text{NO}_3^-$ rates and the impacts of growth medium on leaf counts

Growth media		Outer leaf count (Number)	Inner leaf count (Number)	Total leaf count (Number)
Substrates	Cocopeat	26.00 ± 0.91 b	33.83 ± 0.91 a	59.83 ± 1.31 a
	Peat	23.67 ± 0.70 b	32.00 ± 1.22 a	55.67 ± 1.28 b
	Bark	23.75 ± 0.77 b	31.83 ± 1.34 a	55.58 ± 1.45 b
	Perlite	25.33 ± 0.86 b	32.25 ± 0.98 a	57.58 ± 1.28 ab
	Rockwool	30.50 ± 1.47 a	28.33 ± 2.36 b	58.83 ± 1.41 ab
	LSD	*3.612	*3.192	**3.465
$\text{NH}_4^+:\text{NO}_3^-$	0:100	26.00 ± 1.19 ab	29.13 ± 1.03 b	55.13 ± 0.75 b
	20:80	24.20 ± 0.66 b	30.87 ± 0.93 ab	55.07 ± 1.06 b
	40:60	25.00 ± 1.39 b	32.00 ± 1.86 ab	57.00 ± 1.04 b
	60:40	28.20 ± 1.56 a	34.60 ± 1.52 a	62.80 ± 0.85 a
		LSD	*2.414	*3.820

Different letters indicate significant differences at $P < 0.05$ by LSD test, * and ** represent $P < 0.05$ and $P < 0.01$, respectively. NS: Not significant. Data are shown as the mean values ± SE (for substrates $n=12$ and for nitrate ratios $n=15$).

Table 4. Various $\text{NH}_4^+:\text{NO}_3^-$ rates and the impacts of growth medium on leaf fresh and dry weight and dry matter rate

Growth media		Leaf fresh weight (g)	Leaf dry weight (g)	Dry matter rate (%)
Substrates	Cocopeat	529.17 ± 35.05 a	25.84 ± 1.20 ab	4.94 ± 0.18 b
	Peat	543.83 ± 18.98 a	27.57 ± 1.12 a	5.08 ± 0.13 b
	Bark	373.50 ± 12.77 c	19.10 ± 0.44 c	5.16 ± 0.14 b
	Perlite	466.42 ± 15.98 b	24.42 ± 0.91 b	5.24 ± 0.11 b
	Rockwool	292.50 ± 8.17 d	18.97 ± 0.62 c	6.55 ± 0.30 a
	LSD	**36.680	**2.100	**0.440
$\text{NH}_4^+:\text{NO}_3^-$	0:100	447.67 ± 28.51 a	22.39 ± 1.08 b	5.11 ± 0.16 b
	20:80	451.67 ± 28.95 a	22.71 ± 1.33 b	5.07 ± 0.10 b
	40:60	441.13 ± 26.85 a	23.11 ± 0.96 ab	5.40 ± 0.25 b
	60:40	423.87 ± 30.10 a	24.50 ± 1.15 a	5.98 ± 0.26 a
		LSD	NS	*1.404

Different letters indicate significant differences at $P < 0.05$ by LSD test, * and ** represent $P < 0.05$ and $P < 0.01$, respectively. NS: Not significant. Data are shown as the mean values ± SE (for substrates $n=12$ and for nitrate ratios $n=15$).

Different growth media and their interaction with $\text{NH}_4^+:\text{NO}_3^-$ ratio had significant impact on leaf fresh weight; however, $\text{NH}_4^+:\text{NO}_3^-$ ratios were non-significant in this regard. The highest and the lowest values for leaf fresh weight were noted for cocopeat and peat, and rockwool, respectively. Regarding interaction, peat media with 20:80 $\text{NH}_4^+:\text{NO}_3^-$ ratio recorded the highest leaf fresh weight, whereas rockwool with 0:100, 40:60 and 60:40 ratios resulted in the lowest leaf fresh weight (Figure 2D).

The individual and interactive effects of growth media and $\text{NH}_4^+:\text{NO}_3^-$ ratios significantly altered leaf dry weight. The highest leaf dry weight was noted for peat medium, whereas rockwool and bark media resulted in the lowest dry weigh. The highest and the lowest values for leaf dry weight were obtained from 60:40 and 20:80 and 0:100 $\text{NH}_4^+:\text{NO}_3^-$ ratios, respectively. Regarding interaction, peat with 60:40 and 20:80 $\text{NH}_4^+:\text{NO}_3^-$ ratios recorded the highest leaf dry weight, rockwool with 0:100 $\text{NH}_4^+:\text{NO}_3^-$ resulted in the lowest values (Figure 2E).

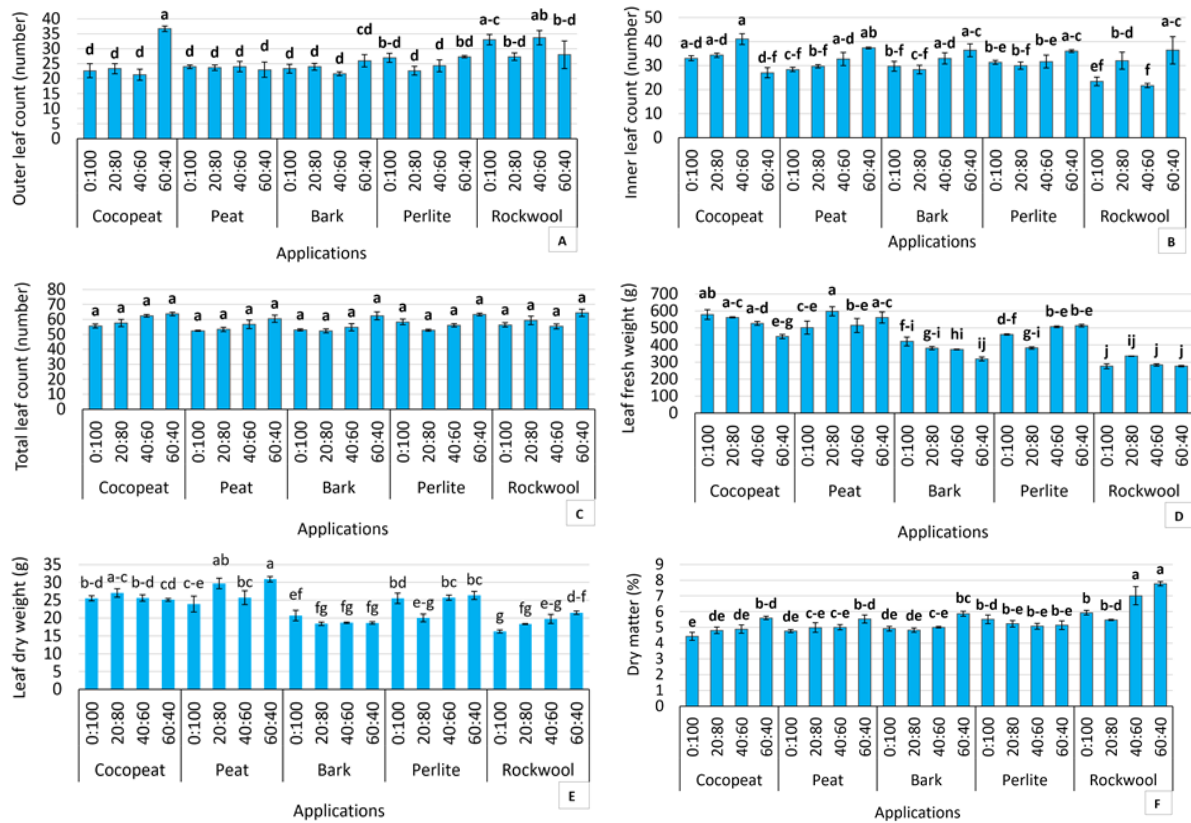


Figure 2. Interactive effect of growth media and $\text{NH}_4^+:\text{NO}_3^-$ ratios on the (A) outer leaf count, (B) inner leaf count, (C) total leaf count, (D) leaf fresh weight, (E) leaf dry weight and (F) dry matter. Data are shown as the mean values \pm SE (n=3). Different letters above the bars indicate significant differences between the treatment ($P < 0.05$, LSD test).

Dry matter ration was significantly affected by individual and interactive effects of growth media and $\text{NH}_4^+:\text{NO}_3^-$ ratios. The highest dry matter ratio was recorded for rockwool medium. Similarly, 60:40 $\text{NH}_4^+:\text{NO}_3^-$ ratios resulted in the highest dry matter ratio. Dry matter ratio decreased with increasing NO_3^- ratio in the nutrient solution. Rockwool with 60:40 and 40:60 $\text{NH}_4^+:\text{NO}_3^-$ ratios recorded the highest dry matter ratio, whereas cocopeat with 0:100 ratio resulted in the lowest values of dry matter ratio (Figure 2F).

Stem and root traits

The effects of growing media and $\text{NH}_4^+:\text{NO}_3^-$ ratios on stem length were significant. Plants grown in peat medium had the longest stem length, while bark and rockwool medium gave the lowest stem length. It was observed that the application with the $\text{NH}_4^+:\text{NO}_3^-$ ratio of 60:40 produced shorter stem length compared to the other applications. The longest stem plants were obtained from the 60:40 $\text{NH}_4^+:\text{NO}_3^-$ application in peat medium, and the shortest stem from the 40:60 $\text{NH}_4^+:\text{NO}_3^-$ ratio in bark medium (Figure 3A).

Stem fresh weight was significantly affected by growth media. However, $\text{NH}_4^+:\text{NO}_3^-$ ratio and interactive effect of growth media and $\text{NH}_4^+:\text{NO}_3^-$ remained non-significant (Table 5). The highest values of stem fresh weight were noted for peat, cocopeat and perlite media, whereas bark and rockwool media resulted in the lowest values. Peat medium with 60:40 $\text{NH}_4^+:\text{NO}_3^-$ ratio resulted in the highest stem fresh weight, while bark medium with 60:40 ratio resulted in the lowest stem fresh weight (Figure 3B).

Table 5. Various $\text{NH}_4^+:\text{NO}_3^-$ rates and the impacts of growth medium on stem length, stem fresh and dry weight, root dry weight and root length

Growth media		Stem length (mm)	Stem fresh weight (g)	Stem dry weight (g)	Root dry weight (g)	Root length (cm)
Substrates	Cocopeat	66.17 ± 3.05 b	38.81 ± 2.65 a	3.39 ± 0.18 a	5.37 ± 0.54 a	46.83 ± 2.62 ab
	Peat	75.83 ± 2.70 a	39.56 ± 1.90 a	3.22 ± 0.18 ab	3.80 ± 0.34 bc	43.67 ± 2.31 b
	Bark	56.08 ± 1.61 c	23.51 ± 1.20 b	1.96 ± 0.17 d	4.02 ± 0.31 b	50.92 ± 1.82 a
	Perlite	69.55 ± 2.14 b	35.18 ± 1.80 a	2.66 ± 0.22 c	3.37 ± 0.23 c	45.42 ± 2.53 b
	Rockwool	59.83 ± 1.36 c	23.99 ± 0.71 b	2.80 ± 0.16 bc	-	-
LSD		**4.918	**4.859	**0.471	**0.611	*4.237
$\text{NH}_4^+:\text{NO}_3^-$	0:100	67.17 ± 2.31 a	32.77 ± 1.98 a	2.53 ± 0.13 b	2.59 ± 0.13 b	43.58 ± 1.73 c
	20:80	65.53 ± 2.07 ab	31.96 ± 2.20 a	2.44 ± 0.16 b	4.70 ± 0.53 a	45.08 ± 3.22 bc
	40:60	66.87 ± 2.99 a	31.51 ± 2.05 a	3.08 ± 0.23 a	4.77 ± 0.37 a	47.83 ± 1.89 ab
	60:40	62.40 ± 2.78 b	32.59 ± 2.67 a	3.17 ± 0.23 a	4.49 ± 0.24 a	50.33 ± 1.68 a
LSD		*3.287	NS	**0.421	**0.611	*4.237

Different letters indicate significant differences at $P < 0.05$ by LSD test, * and ** represent $P < 0.05$ and $P < 0.01$, respectively. NS: Not significant. Data are shown as the mean values ± SE (for substrates $n=12$ and for nitrate ratios $n=15$).

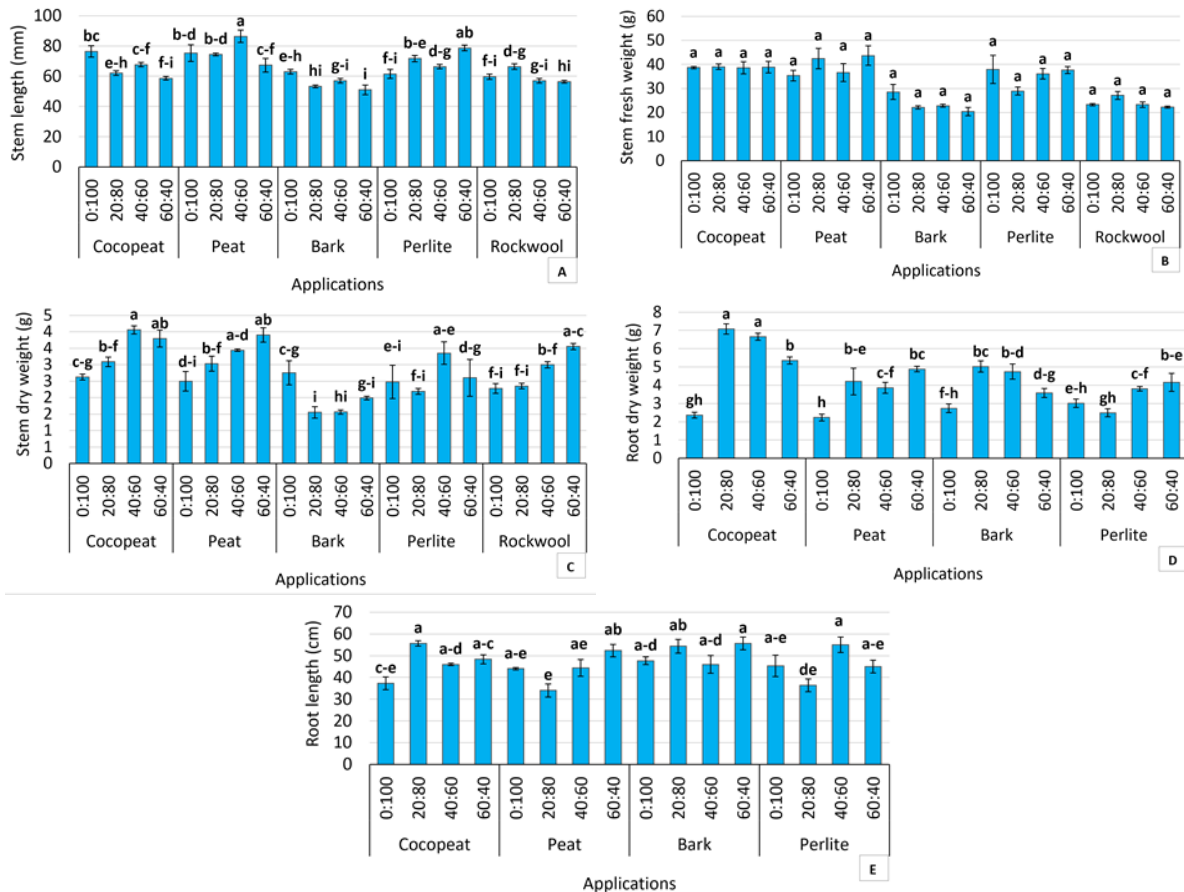


Figure 3. Interactive effect of growth media and $\text{NH}_4^+:\text{NO}_3^-$ ratios on the (A) stem length, (B) stem fresh weight, (C) stem dry weight, (D) root dry weight and (E) root length
Data are shown as the mean values ± SE ($n=3$). Different letters above the bars indicate significant differences between the treatment ($P < 0.05$, LSD test).

Different growth media, $\text{NH}_4^+:\text{NO}_3^-$ ratios and their interaction had significant effect on root dry weight. The highest and the lowest dry weight was noted for cocopeat and perlite media, respectively. Increasing NH_4^+ and decreasing NO_3^- ratio in nutrient solution increased root dry weight. The lowest root dry weight was recorded for 0:100 $\text{NH}_4^+:\text{NO}_3^-$ ratio. Regarding interaction, cocopeat medium with 20:80 and 40:60 $\text{NH}_4^+:\text{NO}_3^-$ ratio recorded the highest root dry weight, while peat medium with 0:100 ratio resulted in the lowest root dry weight (Figure 3D).

Growth media, $\text{NH}_4^+:\text{NO}_3^-$ ratio and their interaction significantly altered root length. The bark medium resulted in the longest roots, whereas the shortest roots were recorded for peat and perlite media. Similarly, the longest and the shortest roots were noted for 60:40 and 0:100 $\text{NH}_4^+:\text{NO}_3^-$ ratio, respectively (Figure 3E).

Discussion

This study examined the impacts of various growth media and $\text{NH}_4^+:\text{NO}_3^-$ ratios in nutrient solution on plant growth and NO_3^- accumulation in lettuce. Growth media significantly affects plant morphology and biomass accumulation (Hahne and Schuch, 2006). Since NH_4^+ and NO_3^- are two main sources of N absorbed by plant roots, it can be argued that N significantly impacts growth and chemical constituents of plants, including vegetables (Therios and Sakellariadis, 1988; Abu-Rayyan *et al.*, 2004; Wang and Li, 2004). The impact of these two forms of N on plant growth depends on plant species and concentrations of these two (Assimakopoulou *et al.*, 2019). Many crops, including vegetables, can use both NO_3^- -N and NH_4^- -N. Where they use less NH_4^- -N, this is usually because there is less NH_4^- -N in the soil (Vargas *et al.*, 2020). The $\text{NH}_4^+:\text{NO}_3^-$ ratio can be used as a strategy to eliminate abiotic stresses such as low light availability, salinity, drought, and heat. Numerous studies have suggested that growing plants with only NH_4^+ or NO_3^- with various $\text{NH}_4^+:\text{NO}_3^-$ ratios can affect tolerance of plants low light availability (Raza *et al.*, 2021), water stress (Shang and Shen, 2018) or salinity (Meng *et al.*, 2016; Ashraf *et al.*, 2021).

In this study, NO_3^- accumulation and head weight were higher in peat and cocopeat media than rest of the media. Head diameter was the highest in peat and perlite media, and head length was the highest in peat and bark media. However, the lowest values for head weight, diameter, and head length were noted on rockwool medium. The reason for a heavier head weight in cocopeat and peat media could be their higher water holding capacity, richer water content, and a lower dry matter rate.

Increased NH_4^+ level in nutrient solution decreased NO_3^- accumulation. The lowest NO_3^- accumulation was obtained for 60% NH_4^+ and 40% NO_3^- . Although impact of $\text{NH}_4^+:\text{NO}_3^-$ ratio was non-significant on head weight, decreasing NO_3^- level in the medium caused a decrease in head weight. It was also observed that low NH_4^+ level improved plant growth. Moreover, decreased NO_3^- level in nutrient solution increased head diameter; however, decreased head length.

Lettuce is one of the vegetables having high NO_3^- level (Ayaz and Yurttagül, 2006). European Commission sets acceptable maximum NO_3^- level as 4000-4500 mg kg^{-1} (fresh weight) for winter lettuce and 2500-3500 mg kg^{-1} (fresh weight) for summer lettuce (Anonymous, 2006). However, it difficult to keep NO_3^- under maximum limit point in regions with low light availability. It is considered that proper $\text{NH}_4^+:\text{NO}_3^-$ ratio in growth media could control NO_3^- level in these regions. The NO_3^- content ranged from 681.00 mg kg^{-1} (bark, 60:40 $\text{NH}_4^+:\text{NO}_3^-$) to 2995 mg kg^{-1} (cocopeat, 0:100 $\text{NH}_4^+:\text{NO}_3^-$) in the current study. It was recorded that if $\text{NH}_4^+:\text{NO}_3^-$ ratio was merely changed, huge differences in NO_3^- accumulation would be detected without much yield loss. However, all these levels were within acceptable range. Several studies suggested that a decrease in NO_3^- levels in nutrient solution decreases NO_3^- accumulation in leaves (Marsic and Osvald, 2002; Wang and Shen, 2011; Shang and Shen 2018). In studies on lettuce and iceberg, increasing N doses increased total N and NO_3^- levels (Mordoğan *et al.*, 2001). It is reported that decreased NO_3^- amount in nutrient solution decreases head weight and head length in iceberg (Marsic and Osvald, 2002). Similarly, Assimakopoulou *et al.* (2019)

reported that $\text{NH}_4^+:\text{NO}_3^-$ ratios have no significant impact on total plant fresh weight of cabbage in the first 4 weeks of cultivation. However, they reported that 75:25 $\text{NH}_4^+:\text{NO}_3^-$ ratio decreased many growth parameters eight weeks after cultivation. In another study, various $\text{NH}_4^+:\text{NO}_3^-$ ratios applied on tomato plants under saline and non-saline conditions significantly affected plant length, yield, and fruit size (Ashraf *et al.*, 2021). Zhao *et al.* (2016) noticed that the impacts of N forms on plant length vary depending on plant species. For instance, while a decrease in $\text{NH}_4^+:\text{NO}_3^-$ ratio caused a shorter plant in pepper (*Capsicum annuum*) (Bar-Tal *et al.*, 2001), it has non-significant impact on plant length in tomato (*Lycopersicon esculentum*) (Sandoval-Villa *et al.*, 2001) and lily (Zhao *et al.*, 2016). On the other hand, another study examining the response of *Saccharum* to changing $\text{NH}_4^+:\text{NO}_3^-$ ratio reported that the lowest photosynthesis was noted where NH_4^+ rate was over 60% (Pissolato *et al.*, 2019). It is suggested that if NH_4^+ is >30% in nutrient solution, photosynthesis and plant growth are disrupted (Pissolato *et al.*, 2019). In another study Chen and Chen (2019) reported that invasive plants grown on only NH_4^+ had lower photosynthetic rate, pigment content and photo system II activity than the plants grown with NO_3^- and NH_4NO_3 . They also concluded that these plants prefer NO_3^- form of N. The plants growing with aerobic respiration often prefer NO_3^- over NH_4^+ . However, swapping partly NO_3^- with NH_4^+ leads to better plant growth (Wang and Shen, 2011). In another study on *Brassica pekinensis*, $\text{NH}_4^+:\text{NO}_3^-$ (10:90) rate increased N metabolism and nitric oxide levels in seedlings, leading to improved tolerance against low light density (Hu *et al.*, 2021). The response of plant species to $\text{NH}_4^+:\text{NO}_3^-$ ratio vary significantly. Generally, dominant NH_4^+ content decreases plant growth as the plants prefer NO_3^- . However, pH of the medium also decides whether plants prefer NO_3^- or NH_4^+ . Moreover, the production of secondary metabolites in plants is affected by the $\text{NH}_4^+:\text{NO}_3^-$ ratio in the nutrient solution. Ammonium or one of its assimilation products can serve as a stress signal, and high ammonium treatment can alter the metabolic pathways of plants, producing more reactive oxygen species (ROS) (Bybordi *et al.*, 2012). Excessive accumulation of ROS can damage lipids, proteins, carbohydrates and nucleic acids in plants (Hatamzadeh *et al.*, 2015) and inhibit the normal growth of plants (Du *et al.*, 2021).

Plants grown in rockwool medium in the current study produced higher number of outer leaves than other growth media. Total leaf count in rockwool were like other media; however, it recorded the lowest leaf fresh weight. As the plants in this medium had a short head length, they had shorter leaves, leading to decreased leaf weight. The highest leaf dry weight was recorded on peat medium. Decreasing NO_3^- rate in solution first slightly decreased outer leaf count, and a dramatic increase was recorded later. The highest inner, outer, and total leaf counts were recorded for 60:40 $\text{NH}_4^+:\text{NO}_3^-$ ratio. However, increase in leaf count did not affect head weight. As the NH_4^+ ratio in nutrient solution increased, leaf dry weight and dry matter rates dramatically increased. The highest leaf dry weight and dry matter rate were obtained from the plants grown on 60:40 $\text{NH}_4^+:\text{NO}_3^-$ ratio. The impact of $\text{NH}_4^+:\text{NO}_3^-$ ratio in nutrient solution might vary depending on plant species. For instance, leaf dry weight is not affected by $\text{NH}_4^+:\text{NO}_3^-$ ratio in lily (Zhao *et al.*, 2016). However, in young velvet mesquite (*Prosopis velutina*), when NH_4^+ is 50% or more, biomass increases (Hahne and Schuch, 2006). For iceberg, it is reported that head fresh and dry weights decrease when NH_4^+ ratio increases (Marsic and Osvald, 2002). In a study on blueberry, it is illustrated that the highest shoot length, leaf count, and plant fresh and dry weights were noted for 5:1 $\text{NH}_4^+:\text{NO}_3^-$ ratio (Yuan-Yuan *et al.*, 2021). In another study on cabbage, it is reported that $\text{NH}_4^+:\text{NO}_3^-$ ratio has no impact on leaf count (Assimakopoulou *et al.*, 2019). Raza *et al.* (2021) demonstrate that only NH_4^+ or NO_3^- applied to soybeans under normal or low light availability, leaf fresh and dry weights decreased. However, they also reported that if both N forms are applied together, these levels increase. In another study on the impact of $\text{NH}_4^+:\text{NO}_3^-$ ratio on the growth of *Brassica chinensis*, it is reported that the highest biomass was obtained from 5:10 $\text{NH}_4^+:\text{NO}_3^-$ ratio, while the lowest biomass value was recorded for 10:5 ratio (Shang and Shen, 2018).

In this study, stem fresh weights on cocopeat, peat and perlite media were higher than other media. The stem dry weights of the plants in cocopeat and stem length of the plant in peat were higher than the plants grown on other media. A decrease in NO_3^- ratio of nutrient solution did not affect stem fresh weight, whereas increasing NH_4^+ ratio affected stem dry weight. The increase in stem dry weight can be explained with

decreasing NO_3^- ratio or increasing dry matter rates in the growth medium. The highest stem length was obtained from 0:100 and 40:60 $\text{NH}_4^+:\text{NO}_3^-$ ratios. Assimakopoulou *et al.* (2019) reported that increasing NH_4^+ in the growing medium leads to a decrease in stem fresh weight and stem length. These findings are in parallel with our study.

Root dry weights of the plants grown in cocopeat were considerably higher than the plants grown on other media. The plants grown on bark media recorded the longest roots. Adding NH_4^+ to nutrient solution increased root dry weight. The experiment in which N in the nutrient solution consisted of only NO_3^- (100%) recorded the lowest root dry weights. Increasing NH_4^+ concentration in the growth medium increased root length as well. The plants grown on the lowest NO_3^- ratio (40%) recorded the longest roots. Likewise, low NO_3^- content increased root dry weight in iceberg (Marsic and Osvald, 2002) and cabbage (Assimakopoulou *et al.*, 2019). Similarly, in another study on lettuce, the highest root dry weights were noted with 75:25 proportioned $\text{NH}_4^+:\text{NO}_3^-$ ratio (Wang and Shen, 2011). In another study on pepper, the longest root and the highest root dry weights was recorded with 25:75 $\text{NH}_4^+:\text{NO}_3^-$ ratio (Zhang *et al.*, 2019). Du *et al.* (2021) obtained highest root dry weight in lettuce with $\text{NH}_4^+:\text{NO}_3^-$ (50:50) or (75:25) ratios.

Conclusions

A proper fertilization is vital for increasing yield and quality of crop plants. The present study revealed that $\text{NH}_4^+:\text{NO}_3^-$ ratios and growth media significantly affected plant growth and NO_3^- deposition. Lettuce plants grow better under stable N availability if NH_4^+ is added as N source compared to NO_3^- -only applications. An increase in NH_4^+ ratio in the solution decreases NO_3^- accumulation by suppressing it. This illustrates that a proper $\text{NH}_4^+:\text{NO}_3^-$ ratio in the solution might support plant growth and decrease NO_3^- accumulation in lettuce. It is hoped that findings of the current study may help in determining an appropriate $\text{NH}_4^+:\text{NO}_3^-$ ratio and growth medium for decreasing NO_3^- accumulation in soilless agriculture.

Authors' Contributions

The experimental design, experiments and data analysis of this article, preparation according to the journal writing rules, and editing works were carried out by SS. The author read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abu-Rayyan A, Kharawish BH, Al-Ismael K (2004). Nitrate content in lettuce (*Lactuca sativa* L.) heads in relation to plant spacing, nitrogen form and irrigation level. *Journal of the Science of Food and Agriculture* 84(9):931-936. <https://doi.org/10.1002/jsfa.1733>
- Aköz G (1981). Besin maddelerinde nitrat ve nitrit içeriğinin saptanması. MSc Dissertation, University of Hacettepe. (in Turkish).
- Anonymous (2006). Setting maximum levels for certain contaminants in foodstuffs. Retrieved from Official Journal of the European Union L 364/5: Retrieved 10 June 2021 from <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF>
- Ashraf M, Naz U, Abid M, Shahzad SM, Aziz A, Akhtar N, Mühling KH (2021). Salinity resistance as a function of $\text{NH}_4^+:\text{NO}_3^-$ ratio and its impact on yield and quality of tomato (*Solanum lycopersicum* L.). *Journal of Plant Nutrition and Soil Science* 184:246-254. <https://doi.org/10.1002/jpln.202000257>
- Assimakopoulou A, Salmas I, Kounavis N, Bastas AI, Michopoulou V, Michail E (2019). The Impact of ammonium to nitrate ratio on the growth and nutritional status of Kale. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 47(3):848-859. <https://doi.org/10.15835/nbha47311466>
- Ayaz A, Yurttagül M (2006). The factors effecting the nitrate and nitrite contents of vegetables. *Journal of Nutrition and Dietetics* 34(2):51-64 (in Turkish). <https://www.beslenmevediyetdergisi.org>
- Bar-Tal A, Aloni B, Karni L, Rosenberg R (2001). Nitrogen nutrition of greenhouse pepper. II. Effects of nitrogen concentration and $\text{NO}_3^-:\text{NH}_4^+$ ratio on growth, transpiration, and nutrient uptake. *HortScience* 36(7):1252-1259. <https://doi.org/10.21273/HORTSCI.36.7.1252>
- Bybordi A, Tabatabaei SJ, Ahmadov A (2012). Influence of salinity and ammonium: Nitrate ratio on growth, photosynthesis, fatty acid and the activity of antioxidative enzymes in canola. *Journal of Plant Nutrition* 35(14):2089-2106. <https://doi.org/10.1080/01904167.2012.723772>
- Cataldo D, Maroon M, Schrader L, Youngs V (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis* 6(1):71-80. <https://doi.org/10.1080/00103627509366547>
- Chen WB, Chen BM (2019). Considering the preferences for nitrogen forms by invasive plants: a case study from a hydroponic culture experiment. *Weed Research* 59:49-57. <https://doi.org/10.1111/wre.12344>
- Çokuyşal B, Anaç D, Eryüce N, Çolak Esetlili B, Özkan CF, Tepecik M (2016). Topraksız Tarım ve bitki besleme teknikleri (1st ed.). In: Anaç D (Ed). Ankara: Nobel Akademik Yayıncılık Eğitim Danışmanlık Tic. Ltd. Şti. (in Turkish).
- Du K, Han Y, Hao J, Qin X, Liu C, Fan S (2021). Effects of different $\text{NO}_3^-:\text{NH}_4^+$ ratios on the ultrastructure and ion flux rate of lettuce roots. *Journal of Plant Nutrition* 44(17):1-18. <https://doi.org/10.1080/01904167.2021.1913184>
- Gül A (2012). Topraksız tarım (2nd ed.). Hasad Yayıncılık (in Turkish).
- Güneş A (1994). The Effect of nitrogenous fertilizers on the growth and nitrate accumulation in spinach grown in Ankara region. PhD Thesis, University of Ankara (in Turkish). <https://tez.yok.gov.tr>
- Hahne KS, Schuch UK (2006). Nitrogen form and concentration affect nitrogen leaching and seedling growth of *Prosopis velutina*. *Hortscience* 41(1):239-243. <https://doi.org/10.21273/HORTSCI.41.1.239>
- Hatamzadeh A, Nalousi AM, Ghasemnezhad M, Biglouei MH (2015). The potential of nitric oxide for reducing oxidative damage induced by drought stress in two turfgrass species, creeping bentgrass and tall fescue. *Grass and Forage Science* 70(3):538-548. <https://doi.org/10.1111/gfs.12135>
- Hu L, Wu Y, Dawuda M, Liao W, Lv J, Li Y, Calderón-Urrea A (2021). Appropriate ammonium/nitrate mitigates low light stress in *Brassica pekinensis* by regulating the nitrogen metabolism and expression levels of key proteins. *Journal of Plant Growth Regulation* 40:574-593. <https://doi.org/10.1007/s00344-020-10113-4>
- Marsic NK, Osvald J (2002). Effects of different nitrogen levels on lettuce growth and nitrate accumulation in iceberg lettuce (*Lactuca sativa* var. *capitata* L.) grown hydroponically under greenhouse conditions. *Gartenbauwissenschaft* 67(4):128-134. <https://www.researchgate.net/publication/287631867>
- Meng S, Su L, Li Y, Wang Y, Zhang C, Zhao Z (2016). Nitrate and ammonium contribute to the distinct nitrogen metabolism of *Populus simonii* during moderate salt stress. *Plos One* 11(3):1-16. <https://doi.org/10.1371/journal.pone.0150354>

- Moncada A, Miceli A, Sabatino L, Iapichino G, D'Anna F, Vetrano F (2018). Effect of molybdenum rate on yield and quality of lettuce, escarole, and curly endive grown in a floating system. *Agronomy* 8(171):1-16. <https://doi.org/10.3390/agronomy8090171>
- Mordoğan N, Ceylan Ş, Çakıcı H, Yoldaş F (2001). Azotlu gübremenin marul bitkisindeki azot birikimine etkisi (*Die Einfluss der N-Düngung über die N-Ansammlung in Kopfsalat*). *Journal of Agriculture Faculty of Ege University* 38(1):85-92 (in Turkish). <https://dergipark.org.tr/en/download/article-file/58934>
- Oruç HH, Ceylan S (2001). Nitrate and nitrite in some vegetables consumed in Bursa. *Journal of Research in Veterinary Medicine* 20:17-21 (in Turkish). <https://dergipark.org.tr/tr/download/article-file/144575>
- Özdehan Ö, Üren A (2010). Nitrate and nitrite in foods. *Academic Food Journal* 8(6):35-43 (in Turkish). <https://dergipark.org.tr/en/download/article-file/1189330>
- Pissolato MD, Silveira NM, Machado EC, Zambrosi FC, Sodek L, Ribeiro RV (2019). Photosynthesis and biomass accumulation in young sugarcane plants grown under increasing ammonium supply in nutrient solution. *Theoretical and Experimental Plant Physiology* 31(3):401-411. <https://doi.org/10.1007/s40626-019-00154-w>
- Rao KP, Rains DW (1976). Nitrate absorption by barley. *Plant Physiology* 57: 59-62. <https://doi.org/10.1104/pp.57.1.55>
- Raza A, Asghar MA, Hussain S, Bin C, Shafiq I, Ahmad I, Weiguo L (2021). Optimal NH₄⁺/NO₃⁻ ratios enhance the shade tolerance of soybean seedlings under low light conditions. *Plant Biology* 23:464-472. <https://doi.org/10.1111/plb.13219>
- Sandoval-Villa M, Guertal EA, Wood CW (2001). Greenhouse tomato response to low ammonium-nitrogen concentrations and duration of ammonium-nitrogen supply. *Journal of Plant Nutrition* 24(11):1787-1798. <https://doi.org/10.1081/PLN-100107312>
- Shang H, Shen G (2018). Effect of ammonium/nitrate ratio on pak choi (*Brassica chinensis* L.) photosynthetic capacity and biomass accumulation under low light intensity and water deficit. *Photosynthetica* 56(4):1039-1046. <https://doi.org/10.1007/s11099-018-0815-7>
- Stepowska A, Kowalczyk W (2001). The effect of growing media on yield and nitrate concentration in lettuce (*Lactuca sativa* var. *capitata* L.). *Acta Horticulturae* 5485:03-510. <https://doi.org/10.17660/ActaHortic.2001.548.60>
- Therios IN, Sakellariadis SD (1988). Effects of nitrogen form on growth and mineral composition of olive plants (*Olea europaea* L.). *Scientia Horticulturae* 35:167-177. [https://doi.org/10.1016/0304-4238\(88\)90110-0](https://doi.org/10.1016/0304-4238(88)90110-0)
- Vargas TD, Concilio A, Woyann LG, Santos RHS, Cheng W (2020). Rhizosphere priming effect on N mineralization in vegetable and grain crop systems. *Plant Soil* 452:281-293. <https://doi.org/10.1007/s11104-020-04566-5>
- Wang B, Shen Q (2011). NH₄-N/NO₃-N ratios on growth and NO₃-N remobilization in root vacuoles and cytoplasm of lettuce genotypes. *Canadian Journal of Plant Science* 91:411-417. <https://doi.org/10.4141/CJPS10044>
- Yardımlı P (2009). Comparison of Kjeldahl and microwave wet digestion methods for determination of total nitrogen content in soil and plant samples. MSc Dissertation, University of Çukurova (in Turkish). <https://tez.yok.gov.tr>
- Yuan-Yuan Z, Jun-Ping T, Jun C, Yu-Hui H, Yu-Shi L (2021). Effects of different NH₄⁺/NO₃⁻ ratios on the photosynthetic and physiology responses of blueberry (*Vaccinium* spp.) seedlings growth. *Journal of Plant Nutrition* 44(6):854-864. <https://doi.org/10.1080/01904167.2021.1871754>
- Wang Z, Li S (2004). Effects of nitrogen and phosphorus fertilization on plant growth and nitrate accumulation in vegetables. *Journal of Plant Nutrition* 27(3):539-556. <https://doi.org/10.1081/PLN-120028877>
- Zandvakili OR, Barker AV, Hashemi M, Etemadi F, Autio WR, Weis S (2019). Growth and nutrient and nitrate accumulation of lettuce under different regimes of nitrogen fertilization. *Journal of Plant Nutrition* 42(14):1575-1593. <https://doi.org/10.1080/01904167.2019.1617313>
- Zhang J, Lv J, Dawuda MM, Xie J, Yu J, Li J, Gan Y (2019). Appropriate ammonium-nitrate ratio improves nutrient accumulation and fruit quality in pepper (*Capsicum annuum* L.). *Agronomy* 9:1-21. <https://doi.org/10.3390/agronomy9110683>
- Zhao X, Bi G, Harkess RL, Blythe EK (2016). Effects of different NH₄:NO₃ ratios on growth and nutrition uptake in *Iris germanica* 'Immortality'. *HortScience* 51(8):1045-1049. <https://doi.org/10.21273/HORTSCI.51.8.1045>



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