EFFECT OF NITROGEN ON GROWTH AND NITRATE ACCUMULATION OF SOME LEAFY VEGETABLES

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ABSTRACT

The effects of nitrogen (N) on growth and nitrate (NO_3^-) accumulation of four different leafy vegetable species, chard (*Beta vulgaris* cv. Chard), garden rocket (*Eruca sativa*), lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*) were studied. The experiment was carried out in high plastic tunnel using sand culture. The experimental treatments consisted of 3 levels of N (110, 175 and 240 mg l^{-1}). Each treatment was in 4 replicates. The plants were irrigated with half concentrated Hoagland's solution containing appropriate amount of N for 45 days and then test plants were harvested and separated into roots and leaves. Total fresh weight (g), leaf number, leaf dry matter (%), plant height (cm) and root length (cm) of test plants were determined. Nitrate and total N analyses were performed in leaf samples. The obtained data were evaluated with the least significant difference ($LSD_{0.05}$) between treatments, derived from analysis of variance. The treatments of N increased growth parameters of the plants. Nitrogen contents of the leaves increased significantly. However the treatments did not cause a regular increase in NO_3^- concentration of the leaves of the plants.

Keywords: NO_3^-, growth, *Beta vulgaris* cv. Chard, *Eruca sativa*, *Lactuca sativa, Spinacia oleracea.*

Azotun Bazı Kışlık Sebzelerde Bitki Gelişimine ve Nitrat Birikimine Etkisi

ÖZET

Bu çalışmada azot (N) uygulamasının dört farklı kışlık sebzede, pazı (*Beta vulgaris* cv. Chard), roka (*Eruca sativa*), marul (*Lactuca sativa*) ve ıspanak (*Spinacia oleracea*), bitki gelişimi ve nitrat (NO_3^-) birikimine olan etkisi araştırılmıştır. Deneme yüksek plastik tünel içinde kum kültürü kullanılarak yürütülmüştür. Çalışmada 3 farklı N dozu (110, 175 ve 240 mg l^{-1}) 4 tekrarlı olarak bitkilere uygulanmıştır. Bitkilere, uygun miktarlarda N içeren yarı yarıya seyreltilmiş Hoagland çözeltileri 45 gün boyunca uygulanmış ve daha sonra hasat edilen bitkiler kök ve toprak üstü aksamlarına ayrılmıştır. Bitkilerin toplam yaş ağırlıkları (g), yaprak sayısı, yaprak kuru maddesi (%), bitki boyu (cm) ve kök uzunluğu (cm) belirlenmiştir. Yapraklarda nitrat (NO_3^-) ve toplam N analizleri yapılmıştır. Elde edilen sonuçlara varyans analizi ve ($LSD_{0.05}$) testi uygulanmıştır. Azot uygulaması bitki gelişimini arttırmıştır. Yaprakların N içeriği önemli düzeyde artmıştır. Bununla beraber uygulama tüm bitkilerde yaprakların NO_3^- içeriklerini düzenli bir şekilde arttırmamıştır.

Anahtar sözcükler: nitrat, bitki gelişimi, *Beta vulgaris* cv. Chard, *Eruca sativa*, *Lactuca sativa, Spinacia oleracea.*

INTRODUCTION

Leafy vegetables such as lettuce, spinach, garden rocket and chard are widely grown and consumed in Turkey. In general the producers are small scaled farmers and they produce these plants using high amounts of chemical fertilizers containing nitrate (NO_3^-) form of nitrogen (N). Most of the production is sold in local markets under uncontrolled conditions.

High rates of N application increase the plant NO_3^- content (Mc Call and Willumsen, 1998). The factors affecting NO_3^- accumulation in plants are mainly nutritional, environmental and physiological. Nitrogen fertilization and light intensity have been identified as the major factors that influence the NO_3^- content in vegetables (Cantliffe, 1973).

Using of high NO_3^- containing plants in human diets can cause some serious problems in consumers, especially in infants (Philips, 1968). According to Dich et al. (1996) vegetables supply about 72% to 94% of the total NO_3^-- N to humans from their daily vegetable diets. After entering into the human body, NO_3^- can be reduced to nitrite (NO_2^-) by bacteria and some enzymes existing in the human digestion system. Nitrite then enters the blood system where it combines with the hemoglobin, and oxidizes Fe^{2+} in it to Fe^{3+}. This prevents the blood system from transferring oxygen and induces methaemoglobinemia. More seriously, NO_2^- can form nitrosamine and nitrosamide, well-known cancer-causing compounds, by reacting with amines and amides (Choi, 1985; Walker, 1990). Hence, many countries have determined NO_3^- concentration limits for vegetables. For instance, these concentrations are 791 mg NO_3^- N kg^{-1} for fresh spinach and 1017 mg NO_3^- N kg^{-1} for fresh lettuce in Germany (Schwemmer, 1990). European Union also

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has similar NO_3^- limits for leafy vegetables (SCF, 1995). The aim of this study was to determine the effect of chemical N application on NO_3^- accumulation of some leafy vegetables used widely in human diets.

MATERIALS AND METHODS

Chard (*Beta vulgaris* cv. Chard), garden rocket (*Eruca sativa* cv. Anadolu), lettuce (*Lactuca sativa* cv. İri Kıvırcık 055) and spinach (*Spinacia oleracea* cv. Green Star) were used as test plants in the study. Commercial seeds of the plants were sown under high plastic tunnel by using a growing media prepared from peat, sand and perlite (50%/30%/20%, v/v/v). Fifteen days after the germination (December 1, 2007), the seedlings of the species were transferred to 5-l buckets filled with coarse sand. The experimental treatment of plants was conducted using half-concentrated Hoagland's solution (Hoagland and Arnon, 1950) with 3 different N levels (110, 175 and 240 mg l^{-1}). Since the growing media used in the experiment was sand, control (without N) treatment were not added to the experimental design. Each N treatment consisted of 45 mg NH_4^+ l^{-1} regularly. The rest of the N treatments were completed with NO_3^-. Each treatment was in 4 replicates and 3 plants per bucket. The application of the treatments lasted for 45 days. Then the plants were harvested and separated into roots and leaves. The total fresh weight (g), the leaf number, the leaf dry matter (%), the plant height (cm) and the root length (cm) of the plants were determined.

For NO_3^- and total N analyses, the leaf samples were placed in paper bags and dried in a forced-air oven (Memmert UM 500, Schwabach, Germany) at 70°C for 72 hours. The dried samples were then ground in a stainless steel mill (IKA A 11 Basic, Staufen, Germany). Total N content of the samples were analyzed by Kjeldahl digestion procedure (Kacar, 1972). For this purpose, 0.25 g of the samples was wet digested in a heating digester (Velp Scientifica, DK20, Milano, Italy) and then distilled in a distillation unit (Velp Scientifica, UDK126A, Milano, Italy). Aliquots were titrated by 0.1 N HCl. The results were expressed as the % N in the DM. Nitrate analysis was performed by colorimetric method according to Cataldo et al., (1975). The principal of the method is to extract nitrate-N in plant dry matter by using hot water. For this purpose 0.10 g of the samples was incubated with 10 ml distilled water for 1 h in 45 °C. The samples was centrifuged for 15 minutes in 5000 rpm and filtered. Then 0.5 ml of the filtrates and 1 ml of salicylic acid-sulphuric acid (5%, w/v) solution were put into flasks. After 20 minutes, 9.5 ml of 4 N solution of NaOH were added to the each flasks. The absorbance of nitrate-N at 410 nm was measured in Shimadzu UV-160 A model spectrophotometer using KNO_3 as reference. In order to obtain the NO_3^- content of the samples the results were multiplied with coefficient of 4.42. The results were expressed as the %-No_3^- in the DM. Additionally the NO_3^- concentrations were also given as mg NO_3^- kg^{-1} in fresh weight (FW) in the text.

Analysis of variance (ANOVA) was performed for the experimental data according to Little and Hills (1978). Mean separation was performed with the LSD (Least Significant Difference) test at $P \leq 0.05$.

RESULTS AND DISCUSSION

The effects of the treatments on the total N content, the NO_3^- content and some growth parameters of the experimental plants were given in Table 1, Table 2, Table 3 and Table 4.

The treatments increased the total N contents in the leaves of the plants significantly. The highest total N concentration was determined in the spinach leaves. The plants of the lettuce, the chard and the garden rocket followed this plant respectively.

The treatments increased NO_3^- contents of the plants except the spinach. However the variations were non-significant except the chard plant. Among the experimental plants concentrations of NO_3^- in the chard and the lettuce plants were highest. The plants of the garden rocket and the spinach followed these plants respectively. Increasing N treatments did not cause an increase in NO_3^- concentration of the spinach leaves. Nitrate concentration of this plant was stable. On the other hand, NO_3^- concentration was highest in the second N dose (175 mg l^{-1}) in the garden rocket and the chard plants, in the third N dose (240 mg l^{-1}) in the lettuce plant.

Table 1. Effects of the treatments on total nitrogen content, nitrate content and growth of the garden rocket *(Eruca sativa)* plant

Plant Nutrients and Growth Parameters	Garden Rocket			
	Nitrogen Doses (mg l^{-1})			
	110	175	240	LSD
Total N Content of the Leaves (% DW^{-1})	2.42 b*	2.55 ab	2.61 a	0.15
Nitrate Content of the Leaves (% DW^{-1})	0.49 ns	0.76	0.76	0.27
Total Fresh Weight of the Plant (g)	72.88 ns	91.23	93.93	23.28
Leaf Dry Matter (%)	15.47 a	13.49 b	12.65 b	1.68
Leaf Number	11.60 ns	12.00	13.50	5.90
Plant Height (cm)	15.00 b	18.50 a	16.80 ab	2.53
Root Length (cm)	26.30 ns	26.80	26.80	6.99

• Values are means of 4 replications. Means separations by least significant difference (LSD) at $P \leq 0.05$, ns:nonsignificant.

Table 2. Effects of the treatments on total nitrogen content, nitrate content and growth of the chard *(Beta vulgaris* cv. Chard*)* plant

Plant Nutrients and Growth Parameters	Chard			
	Nitrogen Doses (mg l^{-1})			
	110	175	240	LSD
Total N Content of the Leaves (%)	2.75 b*	3.08 ab	3.56 a	0.54
Nitrate Content of the Leaves (% DW^{-1})	1.09 b	1.82 a	1.81 a	0.309
Total Fresh Weight of the Plant (g)	112.26 b	133.55 a	153.35 a	21.02
Leaf Dry Matter (%)	10.75 ns	10.10	10.41	0.96
Leaf Number	15.00 ns	15.00	17.00	8.40
Plant Height (cm)	21.10 ns	21.90	26.20	5.12
Root Length (cm)	23.90 b	25.30 ab	27.10 a	3.16

- * Values are means of 4 replications. Means separations by least significant difference (LSD) at P≤0.05, ns:nonsignificant.

Table 3. Effects of the treatments on total nitrogen content, nitrate content and growth of the Spinach (*Spinacia oleracea*) plant

Plant Nutrients and Growth Parameters	Spinach			
	Nitrogen Doses (mg l^{-1})			
	110	175	240	LSD
Total N Content of the Leaves (%)	3.59 b*	4.17 a	4.29 a	0.34
Nitrate Content of the Leaves (% DW^{-1})	0.423 ns	0.417	0.422	0.094
Total Fresh Weight of the Plant (g)	59.47 b	73.64 ab	83.88 a	16.16
Leaf Dry Matter (%)	11.79 ns	11.88	12.04	1.40
Leaf Number	17.2 b	18.0 b	23.30 a	3.30
Plant Height (cm)	12.5 b	13.0 b	14.10 a	0.70
Root Length (cm)	20.5 ns	22.30	22.80	5.70

- Values are means of 4 replications. Means separations by least significant difference (LSD) at P≤0.05, ns:nonsignificant.

Table 4. Effects of the treatments on total nitrogen content, nitrate content and growth of the Lettuce (*Lactuca sativa*) plant

Plant Nutrients and Growth Parameters	Lettuce			
	Nitrogen Doses (mg l^{-1})			
	110	175	240	LSD
Total N Content of the Leaves (%)	3.02 c*	3.35 b	3.71 a	0.19
Nitrate Content of the Leaves (% DW^{-1})	1.347 ns	1.47	1.49	0.42
Total Fresh Weight of the Plant (g)	140.70 ns	127.32	125.00	45.14
Leaf Dry Matter (%)	5.76 ns	6.29	6.29	1.09
Leaf Number	15.80 ns	16.00	16.00	4.30
Plant Height (cm)	12.30 ns	13.50	13.30	1.80
Root Length (cm)	22.60 ns	22.80	22.60	5.70

- Values are means of 4 replications. Means separations by least significant difference (LSD) at P≤0.05, ns:nonsignificant.

In general the treatments promoted the growth of the aerial parts and roots of the plants. Total fresh weight of the plants increased except the lettuce plant. Leaf dry matter (%) of the spinach and the lettuce plants increased while leaf dry matter (%) of the garden rocket and the chard plants decreased. The leaf number and the plant height of the plants increased. Root length of the plants increased except the lettuce plant.

There is no any previous published result about the leaf NO_3^- concentrations of the garden rocket and chard plants. However when the results obtained from this study are compared to the data given for other leafy vegetables such as spinach and lettuce in the literature it is seen that the NO_3^- concentrations of the garden rocket (Table 1) and the chard plants (Table 2) are high enough that can be harmful for the consumers. Nitrate concentration limits for fresh spinach is 791 mg NO_3^-N kg^{-1} and for fresh lettuce is 1017 mg NO_3^-N kg^{-1} in Germany (Schwemmer, 1990). In our study the highest NO_3^- concentrations determined for the garden rocket and the chard plants are 0.763% in DW (or 1029.7 mg NO_3^- kg^{-1} in FW) and 1.810% in DW (or 1885.4 mg NO_3^- kg^{-1} in FW) respectively.

Spinach and lettuce are the plants that have been examined frequently in terms of NO_3^- accumulation levels by the researchers. Fytianos and Zarogiannis, (1999) stated that spinach plant contains particularly high NO_3^- concentrations, commonly around 0.1 % NO_3^-, and values in excess of 0.3 % NO_3^- have been reported on several occasions. According to European Commission (EC) Regulation No. 1822 (2005), maximum NO_3^- level that can be obtained by fresh spinach leaves harvested between 1 October and 31 March is 0.3 % NO_3^- (3000 mg NO_3^- kg^{-1}). In our study the highest NO_3^- concentration determined for the spinach (Table 3) is 0.422% in DW (or 508 mg NO_3^- kg^{-1} in FW).

Similarly lettuce is rich in NO_3^- and concentrations approaching 0.6% NO_3^- have been reported (Fytianos and Zarogiannis, 1999). Maximum NO_3^- content accepted by European Commission (2005) is 0.45 % NO_3^- (4500 mg NO_3^- kg^{-1}) for fresh lettuce grown under cover in winter time (1 October to 31 March). In our study the highest NO_3^- concentrations determined for the lettuce plants (Table 4) is 1.493% in DW (or 939.6 mg NO_3^- kg^{-1} in FW). Nitrate analyses performed in lettuce sold in common supermarkets in Aydın, Turkey have also showed that mean NO_3^- concentrations of this plants' leaves is about 925 mg NO_3^- kg^{-1} in FW (or 1.55% in DW) (Unpublished data).

There is no any previous published work about the sufficient ranges of N of the garden rocket and the chard plants' leaves. However according to Jones et al., (1991) the total N levels of the spinach and the lettuce leaves were classified as "sufficient" and "insufficient" respectively. In that regard none of the N treatments used in the study caused excessive total N concentrations in the spinach and the lettuce plants' leaves. It is known that total N and NO_3^- accumulations might also be affected by the environmental conditions such as light intensity and weather temperature. For instance according to Anjana et al. (2006), NO_3^- accumulation in plants is affected greatly by environmental factors. Santamaria et al. (2001) observed an interaction between light intensity, N availability and temperature on NO_3^- accumulation in garden rocket. According to the author an increase in temperature increases the NO_3^- accumulation under low light availability conditions. However under high light intensity, an increase in temperature increases the NO_3^- content mainly when the N supply is high. As reported by Chadjaa et al. (2001) high-pressure sodium vapor lamps increased the NO_3^- reductase activity and reduced the NO_3^- accumulation. According to Custic et al. (2003) plant NO_3^- levels were influenced by weather conditions more significantly than by the form and application rates of fertilizers.

In conclusion, the results obtained from the study showed that the leafy vegetables used in the study differed in response to the treatments in the experimental conditions. Although there is no any previous published result about the leaf NO_3^- concentrations of the garden rocket and chard plants, the NO_3^- concentration of these plants' leaves were higher than the NO_3^- limits determined by Germany for the spinach and the lettuce plants. On the other hand the NO_3^- values of the spinach and the lettuce plants' leaves were lower than the NO_3^- limits determined by the European Commission and Germany. In this respect it is concluded that the growing of the spinach and the lettuce plants under high plastic tunnels by using chemical fertilizers of N up to 240 mg N l^{-1} is feasible.

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