The Effects of Nitrogen Fertilization on Yield and Quality of Spinach Grown in High Tunnels

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ABSTRACT

A study was conducted under a high tunnel during the spring season of 2014 at the University of Wyoming Research and Extension Center located in Powell, WY, USA, to investigate the effect of nitrogen (N) fertilization on growth, yield, and quality of spinach. Four N fertilization rates (56, 112, 168 and 224 kg N ha^{-1} as urea) were used in a completely randomized design with three replications. Eight weeks after planting, the spinach were harvested. The results indicated that increasing N fertilization rates up to 224 kg N ha^{-1} increased plant growth (fresh and dry plant weight, leaf area and leaf area index) and yield (fresh and dry weight as ton ha^{-1}). Increasing N fertilization rates significantly decreased spinach quality parameters (moisture and dry matter contents and nitrate content). The nitrogen utilization and use efficiency were decreased significantly with increasing N fertilization rates. Increasing N fertilization rates generally increased the spinach content of N, P, K, Fe, and Cu and decreased the contents of Mn, and did not affect the contents of S, Ca, Mg, and Zn. The results suggest that increasing N fertilization rates up to 224 kg N ha^{-1} under a high tunnel conditions has positive effects on spinach yield and adverse effect on spinach quality.

Keywords: *Spinacia oleracea* L., N fertilization, hoop houses, N uptake and N use efficiency, mineral content.

INTRODUCTION

Spinach (*Spinacia oleracea* L.) is one of the major leafy vegetables, which is widely cultivated in spring, autumn, and winter seasons. Spinach is a good source of vitamins A, B1, B2, and C, as well as minerals such as calcium, iron, and magnesium (Kawazu et al., 2003). The global planting area for spinach is reported to reach 893,494 ha with 14,044,816 tons of spinach production in 2007 (FAO Statistical Yearbook, 2008).

High tunnels, also referred to as hoop houses, are simple plastic-covered and passive solar-heated structures in which crops are grown in the ground or in raised beds. They have become an important tool in commercial vegetable and small fruit production in the Midwest USA (http://www.extension.iastate.edu/agdm/crops/pdf/a1-23.pdf), and have become increasingly popular in locations such as Wyoming, USA where they are used as a method to protect tender plants from unpredictable early and late weather-related risks (frost days, wind, and hail) (http://www.wyomingextension.org). Additionally, producers are able to double the length of the growing season for the production of a wide variety of specialty crops (Bachmann, 2005). Usually, high tunnels are unheated structures for producing vegetables beyond the normal growing season (Gent, 1991; Wells, 1996). The environment variations under these structures are wider as compared to the heated/cooled greenhouses. This may have serious consequences on crop production due to the response of plants to such variations. For example, when tomatoes are planted in high tunnels too early in the spring, the plants develop severe nutrient deficiency due to cold soil (Gent, 1992), evidencing the need for a better understanding of factors affecting crop production in protected environments.

Nitrogen (N) is one of the most important nutrients limiting plant growth (Mengel and Kirkby, 1987), which is evidenced by its high demand for vegetable production. Although the application of N fertilizer can improve the yield of leafy vegetables, its oversupply may cause economic loss and environmental degradation as well as a reduction in food quality (Mozafar, 1993; Peng et al., 1996; Darwish et al. 2006; Zhang et al. 2012). In addition, the over use of N could also increase nitrate concentration in spinach, which may be noxious to both animals and humans (Citak and Sonmez, 2010) as it could lead to the production of potentially carcinogenic compounds called *N*-nitrosamines (van Velzen et al., 2008). The high intake of nitrate through drinking water and food may cause a number of health problems, such as gastrointestinal cancer and methaemoglobinaemia (Bruning-Fann and Kaneene, 1993a, 1993b). Certain vegetables tend to accumulate more nitrates (NO_3^-) than others due to a very efficient uptake system, an inefficient reductive system, or an unfavorable combination of both (Maynard et al., 1976). For instance, spinach is listed as being nitrate accumulator (Maynard et al., 1976). This is confirmed by the National Food Administration (NFA) of Sweden, which categorizes vegetable crops based on their nitrate concentration as high (>1000 mg kg^{-1}; fresh lettuce and spinach), intermediate (350 - 1000 mg kg^{-1}; Chinese cabbage, iceberg lettuce, leek, beetroot, and white cabbage), and low (< 350 mg kg^{-1}; broccoli, cucumbers, carrot, cauliflower, potato, and tomato). Because of its high nitrate accumulation

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Received August 15, 2016, Accepted September 21, 2016

capacity, spinach is of interest from the perspective of human health.

Consumption of vegetables, together with drinking water, is one of the main sources of dietary nitrate (NO_3^-) intake (Speijers, 1998; Santamaria et al., 1999). Alone, our consumption of vegetables represents about 75% of our dietary nitrate intake (Forman et al., 1985; MAFF, 1987). The highest nitrate levels in crops have been found in leafy vegetables, with about 500–1000 mg NO_3^- kg^{-1} of fresh leaves (Karlowski, 1990). These levels are above the reference intake of 420 mg per day for a 60 kg person (7.0 mg kg^{-1} body weight) established by the US-Environmental Protection Agency (Liu et al., 2014) and far above the reference daily intake of 222 mg (3.7 mg kg^{-1} body weight) established by the World Health Organization. Reducing such nitrate levels is highly recommended for human health. Maximum admissible levels of nitrate in leafy vegetables have been set up by European authorities (European Commission, 2002) at 2500 and 3000 mg $NO_3^-kg^{-1}$ for fresh summer and winter spinach, respectively.

Correct N fertilization rates under high tunnels conditions are extremely important since they will ensure an optimum level of yield and quality while maintaining a balance in the agroecosystem. Nitrogen fertilizer application rates are often difficult to determine because of soil moisture (leaching), decomposition (e.g.: denitrification), and volatilization, which is also influenced by the presence of N and its mobility in the soil (Montemurro et al., 2007b). As suggested by Montemurro et al., (2007a), our understanding of soil factors, crop N requirement, N uptake, and N utilization efficiency may help to minimize uncertainties associated with such a complex system. This would allow for the development of strategies to maximize the yield response to N fertilizer. Enhancing the N uptake efficiency of horticultural crops is possible but wide variation have been reported due to either differences in environmental conditions or differences in the yield response to N fertilizer (Novoa and Loomis, 1981). As a result, an efficient use of N is a critical goal in horticultural crop production management.

The objectives of this study were to (a) evaluate the effects of nitrogen fertilization rates on growth, yield, and quality of spinach grown under high tunnels, and (b) determine the nitrogen efficiency of spinach grown under high tunnels.

MATERIALS AND METHODS

During 2014 growing season, Spinach (Spinacia oleracea L., cv. 'Bloomsdale Savoy') seeds were manually sown on April 16th in the soil of an unheated high tunnel located at the University of Wyoming Research and Extension Center (44° 45° lat. N, 108° 45 long. W, and 1333 m above sea level), Powell, WY, USA. The high tunnel (7.3×19.5×2.5 m) was constructed of PVC hoops covered with a single layer of SOLARIG™ 140 woven polyethylene film. Large doors located at each end and windows powered by an automated system which were used for ventilation when the inside air temperature reached 24°C. The maximum and minimum air temperature and mean relative humidity inside and outside the high tunnel used in the study were 41.2°C, -0.8°C, 54.3%, 32.5°C, -1.3°C, and 55.9%, respectively.

The soil in the high tunnel was characterized as loamy (sand 37%, silt 36% and clay 27%) with slightly alkaline reaction (pH 7.9), EC of 1.94 dSm^{-1} and containing 1.6% organic matter, 55.5 ppm nitrate nitrogen, 42 ppm available phosphorus and 595 ppm available potassium. An organic compost was mixed into the soil at depth of about 15 cm at a 1:3 ratio. The analysis of the compost was 48.08% moisture, 34.4:1 C:N ratio, 9.01 pH, EC of 13.68 dSm^{-1}, 1.09% total nitrogen, 0.33% total phosphorus and 1.51% total potassium.

The experiment was set in a completely randomized design with three replications. The N fertilization rates were 56, 112, 168 and 224 kg N ha^{-1} as urea (46-0-0). The N fertilization was top-dressed on two applications after 38 and 51 days from planting. The plants were drip irrigated and fertilized by topdressing with 84 and 56 kg ha^{-1} of P_2O_5 as monoammonium phosphate (11-52-0) and K_2O as potassium sulfate (0-0-46), respectively after 38 days from planting. Each plot was 1.5 × 3 m and the spinach was planted in rows spaced 30 cm apart at the rate of 10 seeds per 30 cm

A 0.3 m^2 area from the middle of each plot was harvested 56 days after planting and weighed. The leaf area index (LAI) was derived from the leaf area of one plant obtained with a LI-3100 Area Meter (LI-COR; Lincoln, NE - USA). Plant samples were collected, washed with tap water then by distilled water and were oven dried for 48 h at 65°C to determine their dry weight. The plant fresh, dry weight, the moisture content, and dry matter content of plant were calculated. The yields (fresh and dry matter weights in t ha^{-1}) were also calculated. The oven-dried plant samples were analyzed for total P, K, S, Ca, Mg, Fe, Zn, Cu and Mn contents according toCampbell and Plank (1991); Kovar (2003) and Wolf et al. (2003). Furthermore, the determination of total N (Gavlak et al., 1996) and nitrate (Lachat Instruments, 1992) contents were carried out, allowing the calculation of total N uptake (N content × dry matter yield). On the basis of these measurements and according to procedures from Delogu et al., (1998), López-Bellido et al., (2005), and Montemurro et al., (2006), the N utilization efficiency (NUtE; ratio of dry matter yield to N uptake, in kg kg^{-1}) and the N use efficiency (NUsE; ratio of dry matter yield to N applied, in kg kg^{-1}) were calculated.

The obtained data were subjected to analysis of variance using the PROC GLM procedure of SAS (SAS Institute, Inc., 2002). The Least significant difference (LSD) test at alpha = 0.05 level of significance was used to separate mean differences (SAS Institute, Inc., 2002). Regression analyses were conducted with SigmaPlot (Systat Software Inc., San Jose, CA).

RESULTS AND DISCUSSIONS

Effect of N fertilization on growth of spinach

Increasing N fertilization rates significantly ($p < 0.01$) increased plant fresh weightin general but there were no significant differences on plant fresh weight when 112 and 168 kg N ha^{-1} were used (Table 1). Also, increasing N fertilization rates significantly ($p < 0.05$) increased plant dry weight but no significant differences were observed in plant dry weight when comparing the effects of N rates 56 and 112, 112 and 168, and 168 and 224 kg N ha^{-1}. The relative increases in plant fresh weight with increasing N fertilization rates were 63.5, 104.7, and 164.8 % at 112, 168, and 224 kg N ha^{-1}, respectively as compared to that of the lowest N fertilization rate (56 kg N ha^{-1}). On the other hand, the relative increases in plant dry weight were 7.5, 36.2 and 55.2 % at 112, 168, and 224 kg N ha^{-1}, respectively as compared to that of the lowest N rate (56 kg N ha^{-1}).

Significant differences ($p < 0.01$) in leaf area were recorded with increasing N fertilization rates. However, N rates of 112 and 168 kg N ha^{-1} provided similar leaf area (Table 1). The relative increases in leaf area with increasing N fertilization rates were 49.7, 69.1, and 119.1 % at 112, 168, and 224 kg N ha^{-1}, respectively as compared to that produced by 56 kg N ha^{-1}. The biomass accumulation has been increased, with increasing nitrogen fertilization rate, mainly due to the increase in leaf area.

Increasing N fertilization rates significantly ($p < 0.01$) increased leaf area index (Table 1) from 1.26 m^2 m^{-2} with N rate of 56 kg N ha^{-1} to 4.06 m^2 m^{-2} at the highest N rate of 224 kg N ha^{-1} while no differences between values of LAI were observed at N rates of 112 and 168 kg N ha^{-1}. The relative increases in leaf area index with increasing N fertilization rates were 93.4, 127.0, and 222.0 % at rates of 112, 168, and 224 kg N ha^{-1}, respectively as compared to that of 56 kg N ha^{-1}. Similar results were obtained by Zhang et al. (2014) who found that increasing nitrogen fertilizer rates increased leaf number, leaf area, plant height, and total above ground biomass of spinach. Kavvadias et al. (2013) found that nitrogen application had significant and positive effects on height and fresh- and dry-matter accumulation in spinach. Gutiérrez-Rodríguez et al. (2013) reported that the total mass, leaf area and dry weight were correlated with changes in total N.

Effect of N fertilization on yield of spinach

Increasing N fertilization rates significantly ($p < 0.01$) increased fresh yield of spinach (Table 2 and Figure 1), but there were no significant differences between 112 and 168 kg N ha^{-1} rates. The relative increase in fresh yield with increasing N fertilization rates were 115.4, 174.1, and 293.5 % at 112, 168, and 224 kg N ha^{-1}, respectively as compared to that of 56 kg N ha^{-1}. The dry yield of spinach increased significantly ($p < 0.01$) with increasing N fertilization rate (Table 2 and Figure 2), but there were no significant differences between 56 and 112 kg N ha^{-1} rates and between 112 and 168 kg N ha^{-1} rates. The relative increases in dry yield with increasing N fertilization rates were 39.4, 81.8, and 128.9 % at rates of 112, 168, and 224 kg N ha^{-1}, respectively as compared to that of 56 kg N ha^{-1}.

It is clear in this study that the fresh yield of spinach is strongly influenced by the rate of N fertilizer applied. Thus, increasing application of N fertilization up to 224 kg N ha^{-1} significantly ($p < 0.01$) increased the fresh and dry yield of spinach.

Table 1. Effect of nitrogen fertilization rates on growth of spinach under high tunnel conditions

Nitrogen rates (kg N ha^{-1})	Plant weight (g $plant^{-1}$)		Leaf area (cm^2 $plant^{-1}$)	LAI (m^2 m^{-2})
	Fresh	Dry		
56	31.39 c*	3.93 c	354.1 c	1.26 c
112	51.31 b	4.22 bc	530.2 b	2.44 b
168	64.28 b	5.35 ab	598.8 b	2.86 b
224	83.14 a	6.10 a	775.9 a	4.06 a

*Within column, means with the same letter are not significantly different according to LSD comparison at the P ≤ 0.05 probability level.

Table 2. Effects of nitrogen rates on yield, moisture, dry matter, and nitrate content of spinach

Nitrogen rates (kg N ha^{-1})	Fresh Yield (t ha^{-1})	Dry Yield	Plant Moisture (%)	Plant Dry Matter	NO_3-N (mg kg^{-1})
56	10.55 c*	1.39 c	86.86 b	12.47 a	268 c
112	22.73 b	1.94 bc	91.30 a	8.32 b	3888 b
168	28.92 b	2.52 b	91.27 a	8.35 b	3187 b
224	41.51 a	3.18 a	92.33 a	7.35 b	11294 a

*Within column, means with the same letter are not significantly different according to LSD comparison at the P ≤ 0.05 probability level.

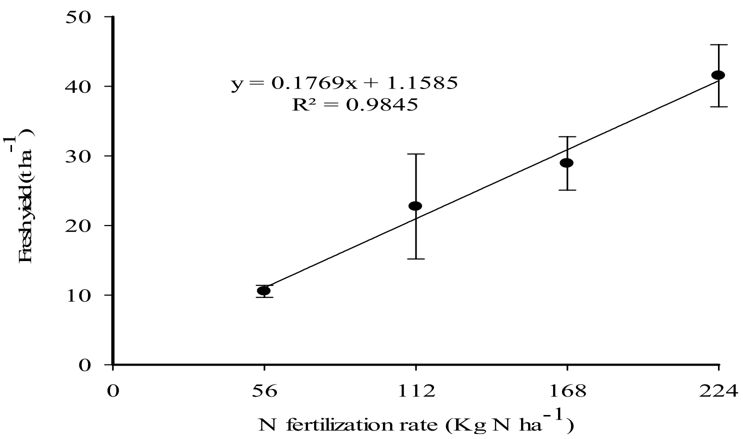

Fig. 1. The relation between N fertilization rates and the fresh yields of spinach grown under high tunnel conditions (vertical lines are standard deviation)

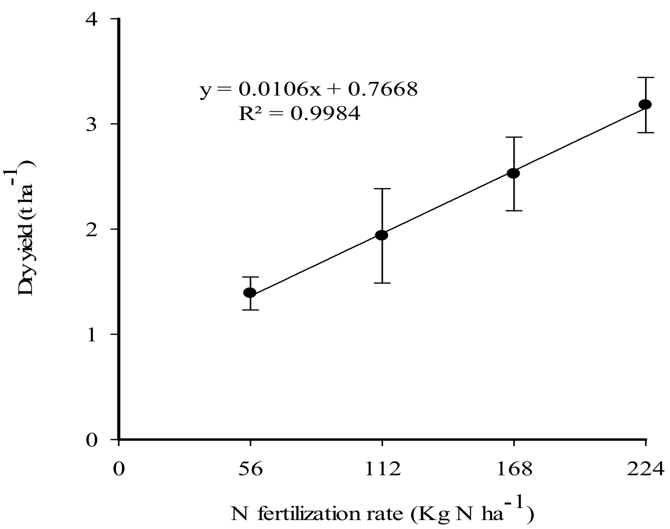

Fig. 2. The relation between N fertilization rates and the dry yields of spinach under high tunnel conditions (vertical lines are standard deviation)

Wang and Li (2004) found that application of ammonium chloride, ammonium nitrate, sodium nitrate, and urea significantly increased the yield of Peking cabbage and spinach. They reported that the vegetable yields were not increased continuously with increasing N application rate and an excess input of N fertilizer, more or less, reduced plant growth thereby leading to yield decline for the earlier harvests. Zhang et al (2014) reported that spinach yields were higher when grown under the condition of soil water content of 16.5% combined with 170 kg ha^{-1} of nitrogen fertilizer.

Effect of N fertilization rates on quality of spinach

Table 2 showed significant differences ($p < 0.01$) in plant moisture content with increasing N fertilization rates. These differences, however, occurred between the lowest N rate and the other N rates, since there were no significant differences between moisture content in plants grown with 112, 168, and 224 kg N ha^{-1} rates. The opposite was observed for dry matter, which showed significant differences ($p < 0.01$) with increasing N fertilization rates (Table 2) between the lowest N rate and the other rates but no significant differences were observed between 112, 168, and 224 kg N ha^{-1} rates. It has been reported that the dry matter of spinach is influenced by genetic, environmental, and agronomic factors such as N supply, which has been shown that it adversely affects this parameter (Muller and Hippe, 1987; Elia et al., 1998; Gruda, 2005).

Increasing N fertilization rates significantly ($p < 0.01$) increased NO_3^- content of spinach plants (Table 2 and Figure 3). The highest N fertilization rate (224 kg N ha^{-1}) showed the highest NO_3^- concentration (11294 mg kg^{-1}), confirming that N fertilization strongly affects the concentration of NO_3^- in the tissue of leafy vegetables (Elia et al., 1998; Santamaria, 2006). The nitrate concentrations in spinach plants were positively correlated with N fertilization rates, where the Pearson's coefficient of correlation (r) of 0.86 was significant at = 0.1%, indicating that the addition of N fertilizer was the major cause for nitrate accumulation in spinach. While N is a key input to increase spinach yield, the dilemma is that nitrate content increases with increasing N-fertilization and therefore N-content. Therefore, maximum spinach yield cannot be achieved without increasing its nitrate content, which is considered a quality-depressing factor (Bassioni et al. 1980) due to its potential effect on human health. In our study, except for the low N rate (56 kg N ha^{-1}), nitrate content of spinach exceeded by far the recommended limit of 3000 mg NO_3^- kg^{-1} for fresh winter spinach at harvest (Table 2) imposed by the Regulation of European Commission (EC) No 563/2002.

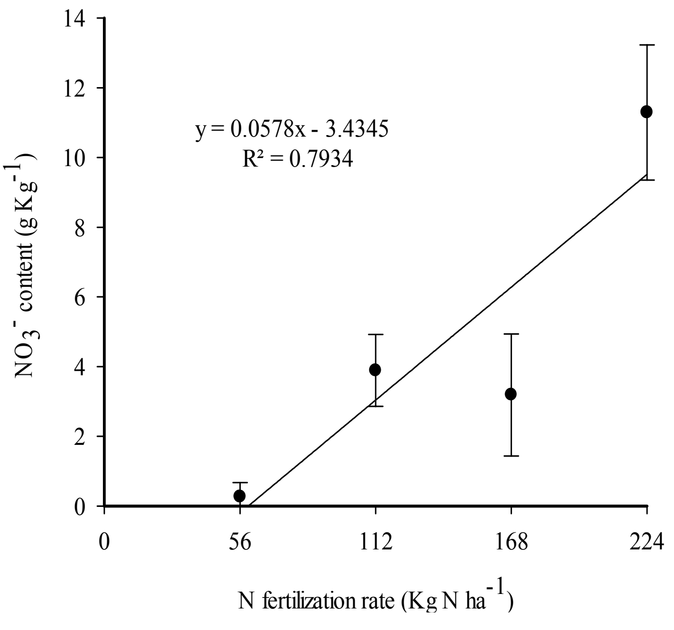

Fig. 3. The relation between N fertilization rates and nitrate content of spinach under high tunnel conditions (vertical lines are standard deviation)

Our results are confirmed by research results in environments where this limit is exceeded in both winter and summer spinach cultivation when a high N fertilization occurs (Kaminishi and Kita, 2006). Similar results were found by Gutiérrez-Rodríguez et al. (2013) who reported a gain in leaf nitrate as the total N supply increased. Mondal and Nad (2012) reported that NO_3-N was increased with increasing N levels but was reduced with phosphorus and sulfur applications and also with advancement in growth.

The high nitrate concentration in spinach may be avoided by delaying the second application of N fertilization to five days before harvest. Applying nitrogen once at the beginning of the cropping cycle is effective at controlling nitrate accumulation, since the plant and soil nitrate concentrations decrease as plants reach a marketable size (Vieira et al., 1998). Some researchers have suggested that the nitrate concentration of leafy vegetables can be lowered to an edible level when harvested within 7 to 8 days after N fertilization (Ren et al., 1997).

Nitrogen uptake and utilization efficiency of spinach

Increasing N fertilization rates significantly ($p < 0.01$) increased the total nitrogen content of spinach (Table 3). The highest total nitrogen content was observed with N rate of 224 kg N ha^{-1} (6 g 100g^{-1}), but there were no significant differences between 112 and 168 kg N ha^{-1} rates. The N uptake was increased significantly ($p < 0.01$) with increasing N fertilization rate but there were no significant differences between N rates of 112 and 168 kg N ha^{-1}. As compared to 56 kg N ha^{-1}, the relative increases in N uptake with increasing N fertilization rates were 134, 166, and 353 % at 112, 168, and 224 kg N ha^{-1}, respectively (Table 3).

Muchow (1998) indicated that maximizing N efficiency parameters requires knowledge of the maximum N requirements for a given yield level, which in turn depends on yield expectation, environmental conditions, management, and cultivars. Our results showed that increasing N fertilizer rates significantly decreased ($p < 0.01$) NUtE and NUsE (Table 3). However, our results showed a higher spinach efficiency in terms of yield when a lower N fertilizer rate (56 kg N ha^{-1}) was applied. These findings are in agreement with those observed by Canali et al. (2011) and Zhang et al. (2014).

It has been reported that increases in N fertilizer rate could lead to increased nitrogen accumulation in spinach and decreased NUtE and NUsE. Nitrogen can be lost through nitrate leaching, denitrification, and ammonia volatilization. Nitrate leaching is the main process through which loss of nitrogen fertilizers occurs. Nitrate leaching losses from soil into water not only reduces soil fertility, but also poses a threat to human health and the environment (Cameron et al., 2013). Reducing nitrate leaching and enhancing NUE are major goals in the development of sustainable agricultural systems. It has been revealed that there is a strong relationship between nitrate leaching and nitrogen fertilizer applications. Therefore, it is necessary to improve nitrogen uptake by plants in order to reduce nitrate leaching (Hu et al., 2010; Min et al., 2011).

Effect of N on minerals content of spinach

The results of the mineral contents in the spinach (Table 4) showed that increasing N fertilization rate generally increased phosphorous content of spinach, but there were no significant differences between N rates; 112 and 224 kg N ha^{-1} and between 56, 112 and 168 kg N ha^{-1} on phosphorous content. The potassium content generally increased with increasing N fertilization rate, but there were no significant differences between N rates of 112 and 224 kg N ha^{-1} and between 112 and 168 kg N ha^{-1} on potassium content in spinach. The highest value of iron content was recorded with N rate of 112 kg N ha^{-1}, but there were no significant differences between 112, 168 and 224 kg N ha^{-1} on iron content in spinach. The manganese content generally decreased with increasing N fertilization rate but there were no significant differences between 112, 168 and 224 kg N ha^{-1} rates on manganese content in spinach. The copper content generally increased with increasing N fertilization rate, but there were no significant differences between N rates of 56 and 168 kg N ha^{-1} and between 112, 168 and 224 kg N ha^{-1} on copper content in spinach. Nitrogen fertilization rates had no significant effect on sulfur, calcium, magnesium and zinc contents in spinach (Table 4).

The Pearson's coefficients of correlation between N fertilization rates and phosphorous, potassium, sulfur, calcium, magnesium, iron, zinc, manganese and copper contents in spinach were 0.54, 0.78, 0.32, 0.64, 0.07, -0.34, 0.52, -0.82 and 0.58, respectively, while between N content in spinach and the same elements were 0.70, 0.93, 0.61, 0.74, 0.37, 0.07, 0.82, -0.83 and 0.84, respectively.

It is clear from these data that N fertilization modified the macro- and micro-nutrient composition of spinach plants and these differences were generally consistent across all experiments. Very close results were found by Gutiérrez-Rodríguez et al. (2013) and Abdel-Razzak et al. (2008). Mondal and Nad (2012) found that the total N, P, K, Ca, Mg, and S uptake were

Table 3. Effect of nitrogen rates on leaf N content, N uptake and N efficiency indices

Nitrogen rates	Total N	N uptake	NUtE	NUsE
(kg N ha^{-1})	(g 100g^{-1} DM)	(kg ha^{-1})	(kg kg^{-1})	
٥٦	3.1 c*	42.9 c	32.8 a	24.8 a
١١٢	5.1 b	98.5 b	19.8 b	17.3 b
١٦٨	4.5 b	111.5 b	22.6 b	15.0 b
٢٢٤	6.0 a	190.9 a	16.7 b	14.2 b

*Within column, means with the same letter are not significantly different according to LSD comparison at the $P \leq 0.05$ probability level.

Table 4. Effect of nitrogen fertilization rates on leaf mineral contents

Nitrogen rates	P	K	S	Ca	Mg	Fe	Zn	Mn	Cu
(kg N ha^{-1})	(g 100g^{-1})					(mg kg^{-1})			
٥٦	0.64 b*	7.42 c	0.36	0.78	0.83	100.5 b	104.7	100.5 a	5.7 b
١١٢	0.76 ab	10.59 ab	0.47	0.96	0.83	203.3 a	139.0	59.0 b	8.7 a
١٦٨	0.67 b	9.66 b	0.42	0.99	0.78	157.0 a	89.0	62.3 b	7.0 ab
٢٢٤	0.81 a	11.49 a	0.43	1.02	0.87	184.0 a	86.0	42.0 b	9.1 a

*Within column, means with the same letter are not significantly different according to LSD comparison at the $P \leq 0.05$ probability level.

increased with increasing N application rates as well as with application of sulfur and phosphorus.

CONCLUSION

Increasing application rate of N fertilization up to 224 kg N ha^{-1} increased growth and yield of spinach grown under high tunnel conditions. The nitrate concentrations were noticeably increased by N fertilization, and a positive correlation was found between nitrate concentrations and N fertilization rates. Increasing N fertilizer rates could lead to increased nitrogen accumulation in spinach and a decrease in both nitrogen utilization efficiency and nitrogen use efficiency. It was also found that N fertilization rates modified the concentrations of nitrogen, phosphorous, potassium, iron, copper, manganese, but had no significant effect on the concentrations of sulfur, calcium and magnesium of spinach.

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الملخص العربي

تأثيرات التسميد النيتروجيني على المحصول وجودة السبانخ المزروعة تحت الأنفاق العالية

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أجريت الدراسة تحت نفق عالي خلال موسم ربيع عام ٢٠١٤ في مركز بحوث وإرشاد جامعة وايومنغ بمدينة باول، بولاية وايومنغ، الولايات المتحدة الأمريكية، لبحث تأثير التسميد النيتروجيني على نمو ومحصول وجودة السبانخ. تم استخدام أربعة معدلات تسميد نيتروجيني (٥٦، ١١٢، ١٦٨ و ٢٢٤ كجم نيتروجين هكتار-١ كسماد يوريا) في تصميم عشوائي كامل مع ثلاثة مكررات. تم حصاد السبانخ بعد ثمانية أسابيع من الزراعة. وأشارت النتائج إلى أن زيادة معدلات التسميد النيتروجيني حتى ٢٢٤ كجم نيتروجين هكتار-١ أدى لزيادة النمو (وزن النبات الرطب والجاف، مساحة الورقة ودليل مساحة الورقة) والمحصول (الوزن الطازج والجاف كطن هكتار-١). زيادة معدلات التسميد النيتروجيني أدى إلى انخفاض بشكل ملحوظ معايير الجودة للسبانخ(الرطوبة ونسبة المادة الجافة ومحتوى النترات). وقد انخفض الاستفادة من النيتروجين وكفاءة الاستخدام بشكل كبير مع زيادة معدلات التسميد النيتروجيني. كما أدت زيادة معدلات التسميد النيتروجيني إلى زيادة محتوى السبانخ من النيتروجين، الفوسفور، البوتاسيوم، والحديد، والنحاس، وانخفض محتوى السبانخ من المنجنيز، و لم تؤثر على محتوى السبانخ من الكبريت، الكالسيوم، المغنيسيوم، والزنك. وتشير النتائج إلى أن زيادة التسميد النيتروجيني تحت ظروف الأنفاق العالية بمعدلات حتى ٢٢٤ كجم هكتار-١ كانت جيدة لمحصول السبانخ وليست جيدة لنوعية السبانخ.