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Effect of Cultivars, Harvesting Time and Level of Nitrogen Fertilizer on Nitrate and Nitrite Content, Yield in Romaine Lettuce

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Abstract: In order to evaluate the effect of nitrogen fertilizer levels (0, 60, 120, 180), cultivars (Pich Ahwazi, Pich Varamini) and harvesting time (morning, evening) on yield, nitrate and nitrite accumulation in edible parts of lettuce, a research was conducted in Shahid Chamran University of Ahwaz, Iran. This study was arranged in spilt plot experiment on randomized complete block design base, in three replications. Results indicated that effects of nitrogen fertilizer amount on yield were significant at 1% levels. Highest yield was accomplished when the Pich Ahwazi cultivar received 120 kg N ha⁻¹. There was appositive relation ship between amount of nitrogen fertilizer and level of nitrate and nitrite accumulation in edible parts of lettuce. In two cultivars nitrate and nitrite is stem and of outer leaves were higher than inner leaves. The effect of harvest time on nitrate and nitrite content was significant at 1% level and nitrate and nitrite amount were lower in evening in comparison to morning harvesting. There had significant different in the nitrate content between cultivar and nitrite accumulation in Pich Varamini was higher than Pich Ahwazi but significant different was not seen among cultivars in nitrite accumulation. Therefore, nitrogen fertilizer and harvesting time are major factors on nitrate and nitrite accumulation in lettuce.

Key words: Lettuce, Nitrogen, Nitrate, Nitrite, yield, cultivar, harvest time

INTRODUCTION

Nitrogen is one of essential elements for growth and development plants and in the nutrition of plants plays a significant role. Plants absorb of nitrogen from the soil in the form of nitrates, which are then converted into proteins and other nitrogen-containing substances (Cash *et al.*, 2002). Nitrate content in a plant represents a dynamic balance between rate of absorption, assimilation and translocation (Maynard *et al.*, 1976). In certain conditions, this balance can be disrupted so that the roots will accumulate nitrate faster than the plant can convert the nitrate to protein (Cash *et al.*, 2002). Nitrate accumulation in plant can be hazardous to human health. Because, in the human body nitrate can be reduced to nitrite which may cause methemoglobinemia furthermore, the possible formation of N-nitroso-compounds from nitrite and secondary nitrogen compounds in the human stomach constitutes a risk (Breimer, 1982).

Vegetables are a major source of nitrate and nitrite in human diet (Amr and Hadidi, 2001). It is estimated that they contribute about 85% (Gaugolli *et al.*, 1994) and 14-43% (Cassens, 1995) of the daily dietary intakes of nitrate and nitrite, respectively, in a number of societies.

Leafy vegetables such as spinach, lettuce and celery contain nitrate at significant levels (Maynard *et al.*, 1976). A number of factors influence nitrate and nitrite

accumulation in vegetables such as nitrate supply from the soil, genotype of plants (Blom-Zandstra and Eenink, 1986; Behr and Wiebe, 1989) and environment under which the plants are grown, such as light intensity and photoperiod (Blom-Zandstra and Lampe, 1985; Van der Boon *et al.*, 1990) and temperature (Van der Boon *et al.*, 1990).

Thus, the many investigations carried out for decreasing nitrate and nitrite accumulation in vegetables. Byrns *et al.* (2001) reported that increasing nitrogen rates of soil caused increases nitrate accumulation in lettuce and outer leaves accumulated higher nitrate than inner leaves. The investigations carried out by Tittonell *et al.* (2003) on lettuce showed that water content and nitrate accumulation in leaf tissues increased with nitrogen application. Amr and Hadidi (2000) in comparison vegetable types observed that effect of cultivar was not significant on nitrate and nitrite content in vegetables. The aim of this work was to determine effect of different nitrogen level, cultivar and time harvest an nitrate and nitrite accumulation in lettuce.

MATERIALS AND METHODS

This study was carried out in the experimental field of Agricultural faculty in Shahid Chamran University of Ahwaz, Iran (from October 2005 to March 2006). The

Table 1: Some physical and chemical properties of experimental soil

Parameters	0-30 cm depth	30-60 cm depth
pH	7.400	7.60
EC(ds m ⁻²)	2.900	2.40
Total N (%)	0.028	0.03
P (ppm)	7.100	6.50
K (ppm)	142.000	123.00
Organic matter (%)	0.560	0.53
Texture	Loam clay silty	Loam

EC: Electrical conductivity, N: Nitrogen and P: Available phosphate
K: Available potassium

experimental design was a split plot in randomized complete blocks with 3 replications. The main plots were included four nitrogen levels (0, 60, 120 and 180 kg ha⁻¹) and sub plots were considered two lettuce cultivar (Pich Ahwazi and Pich Varamini) and two harvesting time (evening and morning harvest). Physical and chemical properties of soil taken from 0 to 60 cm depth in the experimental field are given in Table 1. Before planting, phosphorous (P₂O₅) and potassium (K₂O₃) were applied at a rate of 150 and 100 kg ha⁻¹, respectively. Lettuce seeds were sown in chamber on 24 October 2005. Seven weeks after emergence, seedlings of lettuce were planted in a field at the spacing of 40 cm between rows and 30 cm within plants. Nitrogen fertilizer was applied in three different periods (2, 4 and 8 weeks after lettuce transplanting). In different stages, all protections of farming such as irrigating, weeding, were carried out similarly in all treatments. In harvesting time, sampling of plants was carried out in morning and evening, nitrate and nitrite rate in stem, outer leaves and inner leaves were determined by the similar method. Statistical analysis of experimental data was accomplished using the MSTATC software package and the means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Yields: The effect of different levels of nitrogen on yield was significant at 1% level (Table 2). The highest yield in lettuce was obtained as 7 kg m⁻² in 120 kg ha⁻¹ application; the lowest yield was obtained as 2.83 kg m⁻² in the zero nitrogen application. Increasing the N levels of the fertilizers to 120 kg N ha⁻¹ significantly increased yield of lettuce while yield decreased at the highest nitrogen dose. Decreasing yield might be due to toxicity of plant or non attraction of nitrogen by plant that resulted from the consumption of excesses nitrogen fertilizer (Tabatabaie and Malakoutie, 1997).

Shahbazie (2005) reported that by increasing nitrogen level from 0 to 120 kg N ha⁻¹ yield of lettuce increased but between 100, 150, 200 kg N ha⁻¹ were not observed significant difference. Also, Behtash *et al.* (1995) during experimental on cabbage and celery resulted

Table 2: Mean effects of different nitrogen levels and cultivar on the yield of lettuce

Mean	Yield (kg m ⁻²)
N doses (kg N ha⁻¹)	
0	2.83d*
60	5.14c
120	7.00a
180	6.17b
Cultivar	
Pich Ahwazi	5.36a
Pich Varamini	5.21a

*: Means followed by the same letter(s) did not differ significantly at 5% levels of probability

that application of nitrogen fertilizer increased yield in comparison with control treatment but economically yield and best quality obtained in 100 kg ha⁻¹ application. The effects of cultivar, rate of fertilizer and interaction between cultivar and fertilizer application were not significant.

Nitrate: The results of statistical analysis indicated that effect of nitrogen level on nitrate accumulation was significant at 1% level (Table 3). The rate of nitrate accumulation in lettuce increased when the nitrogen rate was raised to 120 kg N ha⁻¹, whereas application of 180 kg N ha⁻¹ was caused reduction of nitrate accumulation thereby decreasing nitrate content might be due to non uptake of nitrogen or non convert ammonium to nitrate as a result of bacteria toxicity are responsible nitrification in soil (Tabatabaie and Malakoutie, 1997). In two cultivars of lettuce obtained highest nitrate accumulations in stem, the lowest nitrate content obtained in inner leaves. A such seem that stem in comparison with other plant organs had activer vacuole or and cytoplasm system, thus higher nitrate accumulated in it (Zarei, 1995). There had significant difference in the nitrate content between cultivars, nitrate accumulation in Pich Varamini was higher than Pich Ahwazi. The investigations carried out by Parente *et al.* (2006) on lettuce showed that tendency to nitrate accumulation is differed among lettuce cultivars. Therefore, type of cultivar has important role on nitrate accumulation in vegetables and the effect of cultivar on nitrate accumulation has determined in often studies (Munzert, 1989; Rostamforouy, 1999; Shahbazie, 2005). Interaction between nitrogen fertilizer rate and cultivar on nitrate accumulation was significant, the lowest nitrate rates in different organs was existed in the control treatment and Pich Ahwazi cultivar, the highest NO₃ content in inner and outer leaves was existed in the nitrogen level of 120 kg N ha⁻¹ and Pich Ahwazi cultivar, in stem was in nitrogen level of 120 kg N ha⁻¹ and Pich Varamini cultivar. The effect of harvesting time on nitrate accumulation was significant at 1% level and nitrate amount was lower in evening in comparison to morning harvesting. Because, during day trough solar

Table 3: Mean effects of different nitrogen levels, cultivar and harvest time on nitrate and nitrite accumulation of lettuce

Mean for	Nitrate (mg kg ⁻¹ dm)			Nitrite (mg kg ⁻¹ dm)		
	Stem	Outer leaves	Inner leaves	Stem	Outer leaves	Inner leaves
N doses (kg N ha⁻¹)						
0	724.01d*	517.89d	344.14b	10.26c	10.08c	6.66d
60	1380.57c	1188.01c	704.84b	19.62b	15.51b	12.75c
120	2994.54a	2744.82a	1720.25	27.08a	26.70a	19.88a
180	2075.51b	2100.36b	1380.85a	27.04a	26.11a	17.94b
Cultivar						
Pich Ahwazi	1613.63b	1571.54b	1021.11a	20.91a	19.59a	13.55a
Pich Varamini	1973.69a	1704.00a	1053.93a	21.10a	19.61a	15.07a
Harvest time						
Morning	2159.91a	2010.94a	1578.22a	25.42a	23.94a	17.26a
Evening	1427.4b	1264.60b	796.82b	16.58b	15.26b	11.36b

*: Means followed by the same letter(s) did not differ significantly at 5% levels of probability

radiation increasing nitrate reductase activity and nitrate assimilation and thereby decreasing nitrate content of plants (Minnoti, 1977; Lorenz, 1978; Maynard and Barker, 1979). In this case has been a reported similar result by Krohn *et al.* (2003), Cardenas-Navarro *et al.* (1999). Interaction between nitrogen fertilizer rate and harvesting time on nitrate amount was significant at 1% level, in different parts was found the highest nitrate amount in three treatment (120 kg N ha⁻¹), morning harvest and the lowest nitrate content was obtained in the control treatment and evening harvest. Also, Interaction cultivars and harvesting time on nitrate accumulation was significant at 1% level. The lowest nitrate content in inner and outer leaves was related to Pich Varamini cultivar and evening harvest, in stem was related to Pich Ahwazi. Cultivar and evening harvest. The highest nitrate content in different organs was Obtained Pich Varamini and morning harvest. The results shown that interaction between fertilizer level, cultivar and harvesting time on nitrate accumulation in outer and inner leaves of lettuce was not statistically significant, whereas interaction each three factors on nitrate content in stem was significant and the highest nitrate level was found in three treatment (120 kg N ha⁻¹), Pich Varamini cultivar and morning harvest, the lowest nitrate level was obtained in control treatment, Pich Ahwazi cultivar and evening harvest.

Nitrite: The effect of fertilizer amount application on nitrite accumulation was significant at 1% level (Table 3). In different organs was the lowest nitrite content related to the control treatment and the highest nitrite accumulation related to the three treatments, however in outer leaves and stem was not significant difference between Three (120 kg N ha⁻¹) and four (180 kg N ha⁻¹) treatment. The similar results were obtained by Gulser (2005) and Ceylan *et al.* (2002). The effect of cultivar and also interaction cultivar and fertilizer level was not significant on nitrite accumulation but in both cultivars, with increasing the nitrogen fertilizer does increased nitrite accumulation in edible parts. A similar results obtained in the investigations conducted by Amr and

Hadidi (2001) and Lorenz (1987). The effect of harvesting time on nitrite content was significant at 1% level and nitrite accumulation was lower in evening in comparison to morning harvesting the interaction of fertilizer amount and harvest time on nitrite accumulation was statistically significant and the highest nitrite amount in stem and inner leaves was existed in three treatment (120 kg N ha⁻¹) and morning harvest, in outer leaves was in control treatment and morning harvesting. In different organs were the lowest nitrite amount in the control treatment and evening harvest. Furthermore, the interaction of cultivar and harvesting time, also levels of nitrogen fertilizer, cultivar and harvesting time were not significant on nitrite accumulation.

CONCLUSIONS

The results indicated that the rate of nitrogen fertilizer was affected on yield and also nitrate and nitrite accumulation in lettuce. Therefore, with increasing the nitrogen application was increased yield, nitrate and nitrite content in lettuce. Nitrate and nitrite concentration in edible parts of lettuce were higher in morning in comparison to evening harvesting. In all treatments, nitrate and nitrite rate were lower than toxic level. It might be due to that, in the Khuzestan province is very low number of cloudy days (sunny hours were 263.1 on last growing month), thus very light intensity caused increasing nitrate assimilation and thereby decreasing nitrate of plant in evaluating nitrate and nitrite, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established Acceptable Daily Intakes (ADI) of nitrate and nitrate as 0-3.7 NO₃ mg kg⁻¹ body weight and 0-0.07 NO₂ mg kg⁻¹ body weight (JECFA, 1995). In 1997, the European Union established the maximum levels for nitrate content in lettuce. Hence, it should be advised to protecting quality and quantity lettuce use nitrogen fertilizer proportional with best yield crop and for preventing undesirable nitrate and nitrite for human health purpose in lettuce, plants harvesting should carry out in the evening. Also, cultivars should use that nitrate and nitrite accumulation in they are low.

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