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RESEARCH ARTICLE

Yield and quality of lettuce (*Lactuca sativa* L.) depending on variety and type of nitrogen fertilizer

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ABSTRACT

The aim of this research was to determine the effect of the application of different types of nitrogen fertilizer in five different varieties of lettuce on yield. The experiment was performed in the open field, in the spring cycle of growing during 2018 and 2019. The size of the base plot was 3 m^2, and the trial was set up in a random block system. The trial covered the application of various forms of nitrogen fertilizers: ammonium sulphate, ammonium nitrate and urea. The applied nitrogen dose is 100 kg/ha (May 10th 2018 and May 8th 2019). Samples for the analysis of vitamin C and nitrate content, as well as yield determination, were carried out at the stage of the technological maturity of lettuce (60-62 days after seedling). The average weight of lettuce, depending on the variety, varied in the range of 211.3 g to 258.7 g. The use of ammonium nitrate resulted in the highest average weight of lettuce head (246.7 g), followed by the use of urea (238.9 g) and the lowest by the use of ammonium sulphate (221.0 g). The nutritional value of lettuce (vitamin C content) shows that depending on the applied form of nitrogen in the fertilizer, the highest content of vitamin C was determined by urea (10.86 mg/100 g), then ammonium sulphate (9.61 mg/100 g), and the lowest by ammonium nitrate (7.38 mg/100 g). The lower content of vitamin C was found in varieties of lettuce with red leaves compared to varieties with green leaves. The accumulation of nitrate in lettuce leaves directly depends on the variety and the applied fertilizer. The highest nitrate content was with ammonium nitrate (2355.6 mg/kg). The accumulation of nitrate in lettuce varieties with red leaves was higher compared to varieties with green leaves.

Keywords: Lettuce, Nitrate, Vitamin C, Yield, Open field

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a leafy green vegetable that is grown in the cold part of the season due to its tolerance to low temperatures. Lettuce has a short life cycle and a large yield. It is consumed fresh, for preparing different types of salads that are used in human nutrition. It is consumed all over the world.

Lettuce is an excellent source of minerals and vitamins, 6-19% of the dry weight is mineral composition. It is highly valued in the human diet due to its high content of calcium, iron and vitamins C and A. Some researches proved that lettuce leaves have the highest calcium content compared to all other vegetable types. It is low in calories and is often the main component of diets for people who want to reduce body weight (Ghimire et al., 2019; Gashaw and Haile 2020). It has a positive effect on the regulation of blood pressure and cholesterol, reduces inflammatory processes and improves digestion and appetite (Sharma 2005). However, large amounts of nitrates accumulated in leafy vegetables, which are translated into nitrites in the human body, can be very harmful to skin health (Catherine et al., 2017). Due to the negative impact of nitrates on human health, the European Union has determined the maximum acceptable level of nitrates in lettuce, which is 2500-4500 mg/kg of fresh weight (Khan et al., 2018). Some studies proved that nitrogen fertilization and light intensity directly affect the nitrate concentration in lettuce leaves. Due to the great influence of nitrogen on the growth rate and total yield of plants, lettuce crops are often excessively fertilized with it (Ahmed et al., 2022). However, when nitrogen fertilization exceeds the real need for it, lettuce plants accumulate it and then it becomes harmful to human health (Baslam et al., 2011; Francisco and Heinrich 2017; Khan et al., 2018). Reducing the dose of plant nutrition with mineral nitrogen or

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using alternative fertilizers, reduces the nitrate accumulation. However, a critical minimum level of mineral nitrogen is necessary to maintain the yield and nutritional quality of lettuce (Haque and Jakhro, 1996; Tsouvaltzis et al., 2020). Nitrate accumulated in lettuce leaves is also influenced by the forms of nitrogen in mineral fertilizers used during cultivation (Bošković-Rakočević et al., 2009; El-Bassyouni, 2016; Khan et al., 2018). Also, an excessive dose of nitrogen fertilizers leads to a decrease in vitamin C in lettuce leaves. Increased doses of nitrogen fertilizers lead to greater uptake of nitrogen, and thus the consumption of acids from the tricarbon cycle for the synthesis of amino acids at the expense of carbohydrates and vitamin C (Malešević et al. 2005; Ahmed et al., 2021; AlBadwawi et al., 2022).

Some studies have indicated that there may also be a genetic influence on nitrogen uptake. In fact, different lettuce cultivars may have different absorption and nitrogen management capabilities (R'him et al. 2022).

This research was conducted to determine the effect of applying different types of nitrogen fertilizer to five different varieties of lettuce on the yield, the total content of nitrates and vitamin C in fresh leaves.

MATERIAL AND METHODS

Plant material

The experiment with five different lettuce varieties (Plenty-green, Kibou-green, Murai-red, Levistro-green and Carmoli-green) was performed in the open field, in spring cycle of growing during 2018 and 2019 on locality Trbušani near Čačak (N43°55'31" E20°19'14", altitude 281 m), Republic of Serbia. Lettuce varieties that are morphologically different (color, leaf shape, head type) were chosen for the experiment, in order to determine as realistically as possible whether there is an influence of the variety (genotype) on the application of treatments. Soil on which the tests were performed was loam type of soil and by its properties was suitable for growing lettuce (Photo 1). The size of the base plot was 3 m^2, and the trial was set up in a random block system with three replications. Sowing of seeds for seedling production was performed in plastic containers on Klasmann substrate, and the planting of 35 days old seedlings was performed on April 15th, 2018 and April 12th 2019 on 25 x 25 cm (16 plants m^{-2}). The trial covered the application of various forms of nitrogen fertilizers: ammonium sulphate (21% N), ammonium-nitrate (34% N) and urea (46% N). The applied nitrogen dose is 100 kg/ha in the intensive growth phase (May 10th 2018 and May 8th 2019). The lettuce was harvested at technological maturity (June 15th 2018 and June 14th 2019), when the yield was measured and leaf samples were taken to determine the content of nitrate and vitamin C.

The air temperature and precipitation during the experiment were measured at the local meteorological station Trbušani.

Chemical analysis included measurement of pH value – potentiometric (in soil/water and soil/KCl 1:2.5 suspension), determination of humus content (Kotzman method), readily available phosphorus and potassium content - Egner-Riehm AL method (colorimetric phosphorus, flame photometric potassium) and nitrate content nitrogen (Bremner method).

Determination of nitrate content

Nitrate content in leaves was determined in 0.03 M solution of acetic acid with the use of Starck modified Bremner distillation method (Nowosielski, 1988).

Determination of vitamin C content

Vitamin C content was analysed by Tillman's titration method. Quantitative determination of L-ascorbic acid is

Photo 1. The open-field experiment of lettuce

based on the reversible ability of oxido-reduction system of ascorbic-dehydroascorbic acid. Titration with the reagent 2,6-dichlorophenolindophenol (Tillman's reagent, TR) was performed in an acidic environment with pH of 4-6. The oxidized form of TR (also serving as an indicator) is dark blue at pH of 5.2. In the presence of ascorbic acid, TR changes into its reduced colourless form. At pH of 4.2 TR is red (acidic environment), and when the whole amount of L-ascorbic acid is oxidized, the very next drop of TR colours the examined solution pink (Cvijović and Aćamović-Đoković, 2005).

Analysis data

The obtained results were processed by the method of analysis of variance (ANOVA) for two - factorial experiment and Fisher LSD test considering fertilizer and variety as factors, separately for each year of research. Statistical data processing was performed in the Statistica program software version 12.0 (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Chemical analysis of the soil shows the optimal pH value (pH/H_2O 7.3, pH/KCl 6.6), good humus supply (4.2%), easy accessible phosphorus (46.8 mg/100g of soil) and potassium (56.3 mg/100 g of soil), as well as nitrate nitrogen (25.7 mg/kg).

Growing lettuce in the open field is directly influenced by air temperature and rainfall during the vegetation cycle. Mean monthly air temperatures by years of research are similar, with slightly lower temperatures measured in 2018 (Table 1), but without a significant impact on the yield and content of nitrates and vitamin C. During 2018, a higher amount of precipitation was measured, especially in May (95.5 mm), when lettuce was fertilized with nitrogen, which resulted in a decrease in the nitrate content in lettuce leaves, because part of the nitrate nitrogen was washed into deeper layers of the soil.

Table 1: The air temperature (°C) and precipitation (mm) during the experiment

Year	Air temperature (°C)			Precipitation (mm)		
	April	May	June	April	May	June
2018	13.1	16.7	22.0	45.5	95.5	47.0
2019	14.2	17.3	24.1	37.0	68.0	38.0

Weight of leaf rossete

Based on the results of the research (Table 2), we conclude that in the first year the highest average weight of leaf rossete was achieved in the variety Plenty (258.7 g), followed by Kibou (251.0 g), Levistro (248.3 g), Murai (246.7 g), and the lowest in the variety Carmoli (224.7 g). In the second year of the experiment, lower values of the average weight of the head were determined and ranged from 211.3 g (Kibou variety) to 235.0 g (Plenty variety). The determined differences in the average weight of the head show that the lettuce varieties with red leaves have smaller heads compared to the varieties with green leaves, which were previously determined by Chohura and Kolota (2009). Less rainfall during the lettuce growing season in the second year of the study reduced the average head weight in all varieties, as head weight depends on growing season, weather conditions and varieties (Santamaria et al., 2001; Koudela and Petříková, 2008).

Regardless to the variety, the application of ammonium nitrate resulted in the highest average weight of lettuce head (246.7 g), than application urea (238.9 g) and the least by applying ammonium sulphate (221.0 g). According to the values of Fischer's test, no significant difference was found in the weight of lettuce head, both between varieties, fertilization methods, and their interaction (Table 2). Percentage of interaction in total variance was 25.17% (2018) and 20.86% (2019), varieties 16.21% (2018) and 9.25% (2019), and the impact of fertilization 16.48% and 12.6% (respectively), represent low (insignificant) values confirmed by the F test. The absence of statistically significant differences in the average weight of the head between the tested variants is a consequence of the application of nitrogen-based fertilizers, which behaved very similarly in the experiment, i.e. it effected the creation of leaf mass. This is a confirmation of the results obtained

Table 2: Weight of leaf rossete (g)

Fertilizer	Plenty		Kibou		Murai		Levistro		Carmoli		Average
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	
Ammonium sulphate	242	208	224	194	247	238	219	214	220	204	221.0
Ammonium nitrate	263	251	275	209	228	216	285	257	238	245	246.7
Urea	271	246	254	231	265	234	241	225	216	206	238.9
Average	258.7	235.0	251.0	211.3	246.7	229.3	248.3	232.0	224.7	218.3	235.5
					F (Fishers')						
Variety	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fertilizer	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

by Sady et al. (1990) who found a slight variation in the yield of lettuce fertilized with nitrate nitrogen and reduced forms of nitrogen. Kowalska (1998) came to similar conclusions when growing tomatoes. Kalisz (2007) found that the role of nitrogen in increasing lettuce yield is very important, while Premuzic et al (2004) showed that by applying a certain type and moderate dose of nitrogen fertilizer, high yields, quality (content of dry matter and vitamin C) and low accumulation of nitrate can be achieved. Chohura and Kołota (2009) achieved similar results by obtaining the highest yield of lettuce using ammonium nitrate, ammonium sulphate and calcium nitrate. El-Bassyouni (2016) states that ammonium sulphate increased the yield of lettuce compared to ammonium nitrate, but without statistical significance.

Vitamin C

Presence of vitamin C in plants can significantly decrease the formation of met haemoglobin (McKnight et al., 1999), so the ration of nitrates and vitamin C in nutrition is very important in diet. Kastori and Petrović (2003) conclude that if vegetables do not contain extremely high amounts of nitrates, but contain vitamin C and other protective substances that prevent the formation of harmful products of nitrate reduction, hence vegetables as a source of nitrates do not pose a danger to human health.

Content of vitamin C varied in wide range from 2.12 mg/100 g to 20.12 mg/100 g depending on variety and applied nitrogen fertilizer (Table 3). The average content of vitamin C shows that lettuce varieties with red leaves had the lowest level of this vitamin as follows: Carmoli (3.06 mg/100 g) and Murai (3.68 mg/100 g). The content of vitamin C in varieties with green leaves was as follows: Plenty (11.12 mg/100 g), Levistro (13.09 mg/100 g) Kibou (15.48 mg/100 g). Values of vitamin C by years were: 10.39-8.18 mg/100 g in 2018 and 2019), respectively.

In the analysis, the variability of vitamin C content was determined in 2018, both for the variety factor and for the fertilization factor. Interaction of these two factors was not significant. In 2019 no statistically justified parameter was determined, both between factors and for interaction of variety and fertilization (Table 3). Since variability between varieties was found in the first year and not in the second year, many authors disagree about the influence of varieties on vitamin C content. However, they all agree that the level of vitamin C mostly depends on genotype (Llorach et al., 2008; Govedarica-Lučić et al., 2014), weather conditions and latitude (Hägg et al., 1994), light intensity (Nicolle et al., 2004), "before and after harvest" factor (Lee and Kader, 2000). Fu et al. (2017) state that in conditions of higher amount of light and lower dose of nitrogen, there is an increase in the content of vitamin C, and a decrease in the content of nitrate in lettuce. The results of vitamin C content in varieties with green leaves (Plenty, Kibou and Levistro) are in accordance with the results of Cintya et al. (2018), who determined the content of vitamin C in varieties with green leaves from 12.24 to 13.93 mg/100 g. Slightly higher values were found in Koudela and Petříková (2008) and Llorach et al. (2008), and these authors obtained a higher content of vitamin C in varieties with green leaves compared to varieties with red leaves, which was confirmed in our research.

The values of vitamin C also depended on the form of nitrogen in the applied fertilizer. On average, the highest content of vitamin C was determined using urea (10.86 mg/100 g), followed by ammonium sulphate (9.61 mg/100 g), and the lowest by the use of ammonium nitrate (7.38 mg/100 g), which is directly related with nitrate content in lettuce leaves. The obtained results confirm the previous research of El-Bassyouni (2016) who obtained a lower content of vitamin C by using ammonium nitrate compared to ammonium sulphate. The content of vitamin C, in addition to the form of applied nitrogen fertilizer, also depends on the dose of nitrogen (Sylvestre et al., 2019). The ratio of statistical significance to the change in vitamin C content in lettuce leaves in 2018 was justified with the use of ammonium nitrate and urea in the ratio of Plenty vs Kibou, while for the use of ammonium sulphate is not significant. Plenty vs Murai ratio showed a difference in the application of all three types of fertilizers to the content of vitamin C, as well as in the ratio Plenty vs Carmoli. There were no statistical differences in relation Plenty

Table 3: Vitamin C content in lettuce (mg/100g fresh weight)

Fertilizer	Plenty		Kibou		Murai		Levistro		Carmoli		Average
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	
Ammonium sulphate	12.88	12.75	16.04	13.05	4.75	3.63	14.80	11.06	4.25	2.85	9.61
Ammonium nitrate	8.94	6.72	14.56	11.44	3.49	2.12	12.49	8.58	3.24	2.27	7.38
Urea	15.22	10.23	20.12	17.62	4.99	3.08	17.35	14.27	2.75	3.02	10.86
Average	12.35	9.90	16.91	14.04	4.41	2.94	14.88	11.30	3.41	2.71	9.28
					F (Fishers')						
Variety	**	ns	**	ns	**	ns	**	ns	**	ns	**
Fertilizer	**	ns	**	ns	**	ns	**	ns	**	ns	**
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

vs Levistro in vitamin C content comparing to applied fertilizer (Table 4).

Nitrate content

The nitrate content in lettuce leaves shows a direct dependence on the variety and the applied nitrogen fertilizer (Table 5). Depending on the variety, in 2018, the average nitrate content ranged from 1183.7 mg/kg (Kibou variety) to 2226.0 mg/kg (Carmoli), while in the second year of research a higher average nitrate content was found (1675.0 mg/kg - Kibou to 2791.0 mg/kg Carmoli). In the analysis of the nitrate content in lettuce leaves in 2018, a significant difference was shown by the factors of variety and fertilizer, while the interaction of these two factors was not statistically significant. In 2019, both factors were significant at the 99% probability level, as well as their interaction (Table 5).

The obtained results show that the nitrate content in lettuce varieties with red leaves was higher compared to varieties with green leaves, which was previously determined by Chohura and Kołota (2009). Ozgen et al. (2014) state that green and red leaf varieties reacted differently to the application of mineral fertilizers, with varieties that have more anthocyanin accumulating a higher concentration of nitrate than other varieties. The average nitrate content, according to the years of research, was higher in 2019 (2285.7 mg/kg) compared to 2018 (1637.9 mg/kg). If we look at the weather conditions in the years of research (Table 1) we can see that in 2019, during the vegetation cycle of lettuce, there was less precipitation compared to 2018, which resulted in reduced nitrate reductase activity and reduced transpiration, as well as slower plant growth and a lower yield, and thus a greater accumulation of nitrate in lettuce leaves. The average air temperature was higher comparing to 2018, which also impacted the nitrate accumulation. According to research by Kastori and Petrović (2003), the most important factors for nitrate accumulation in lettuce are high temperature and low light intensity, which was confirmed by the results of Govedarica-Lučić et al (2014), who determined the average nitrate content by growing Kibou lettuce in the winter cycle, in greenhouse 2176.85 mg/kg, which are significantly higher values compared to our research (1429.4 mg/kg).

Analysing the impact of individual fertilizers, it can be concluded that the highest nitrate content was achieved by using ammonium nitrate (2355.6 mg/kg). The use of urea had a limiting role in the accumulation of nitrate in lettuce leaves (1561.3 mg/kg), as well as the use of ammonium sulphate (1968.5 mg/kg). Bošković-Rakočević et al. (2017) found that the lowest accumulation of nitrate was found in fertilization with urea, then ammonium nitrate, and the highest in fertilization with nitrate fertilizer (even four times less nitrate accumulates in potatoes when fertilizing with ammonium nitrate compared to nitrate fertilizers). Similar results were obtained in the papers Chohura and Kołota (2009) and El-Bassyouni (2016). According to their research, the nitrate content was lower with the use of ammonium sulphates compared to ammonium nitrates, which is in line with the results that show that fertilizing with reduced forms of nitrogen significantly reduces the nitrate content (Kowalska, 1997; Jarosz and Dzida, 2006). Lower nitrate content may be due to other factors such as fertilizer type, soil condition, agro-ecological conditions, light intensity, harvest time (Pavlou et al., 2007). There were no significant differences in 2018in nitrate content between Plenty vs Kibou when applying nitrogen fertilizer in different forms of nitrogen, while in 2019 the nitrate content was highly significant for ammonium-nitrate

Table 4: Table of significance for vitamin C content

Variety vs variety	Plenty vs Kibou		Plenty vs Murai		Plenty vs Levistro		Plenty vs Carmoli	
Year	2018	2019	2018	2019	2018	2019	2018	2019
Ammonium sulphate	ns	ns	**	ns	ns	ns	**	ns
Ammonium nitrate	**	ns	**	ns	ns	ns	**	ns
Urea	**	ns	**	ns	ns	ns	**	ns

Table 5: The nitrate content in lettuce (mg/kg fresh weight)

Fertilizer	Plenty		Kibou		Murai		Levistro		Carmoli		Average
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	
Ammonium sulphate	1359	2266	1157	1405	1954	2799	1603	1986	2280	2876	1968.5
Ammonium nitrate	1818	2618	1408	1968	2457	3286	1488	2324	2743	3446	2355.6
Urea	1042	2042	986	1652	1598	2246	1021	1320	1655	2051	1561.3
Average	1406.3	2308.7	1183.7	1675.0	2003.0	2777.0	1370.7	1876.7	2226.0	2791.0	1961.8
					F (Fishers')						
Variety	**	**	**	**	**	**	**	**	**	**	**
Fertilizer	**	**	**	**	**	**	**	**	**	**	**
Interaction	ns	**	ns	**	ns	**	ns	**	ns	**	ns

Table 6: Table of significance for nitrate content

Variety vs variety	Plenty vs Kibou		Plenty vs Murai		Plenty vs Levistro		Plenty vs Carmoli	
Year	2018	2019	2018	2019	2018	2019	2018	2019
Ammonium sulphate	ns	**	*	**	ns	ns	**	**
Ammonium nitrate	ns	**	**	**	ns	ns	**	**
Urea	ns	*	*	ns	ns	**	**	ns

and ammonium-sulphate. For urea, this difference is at the level of 95% significance. The nitrate content, in the varieties Plenty vs Murai, in both years of research, differed depending on the application of fertilizers, except for the use of urea in 2019, when this difference was statistically small. The difference between the varieties Plenty vs Levistro is somewhat different. There were no significant differences in these varieties regarding the application of nitrogen fertilizer, except in 2019, when applying urea. Differences between Plenty vs Carmoli varieties were similar to those of Plenty vs Murai varieties, where there was no difference only in the use of urea as a nitrogen nutrient in 2019 (Table 6).

The applied form of nitrogen fertilizer and the choice of lettuce variety shows the impact on the total yield accumulation of nitrates and vitamin C in the leaves. It is very important to correctly choose the best source of nitrogen and variety, in order to obtain a nutritionally valuable and health-safe product. It is necessary to report a greater number of experiments in order to properly understand the mechanism of nutrient accumulation substances that are good for human health as well as those that have a harmful effect (nitrates) in lettuce.

CONCLUSION

The average weight of lettuce, depending on the variety, varied in the range of 211.3 g to 258.7 g. The use of ammonium nitrate resulted in the highest average weight of lettuce head (246.7 g), followed by the use of urea (238.9 g) and the lowest by the use of ammonium sulphate (221.0 g).

The nutritional value of lettuce (vitamin C content) shows that, depending on the applied form of nitrogen in the fertilizer, the highest content of vitamin C was determined by urea (10.86 mg/100 g), then ammonium sulphate (9.61 mg/100 g), and the lowest by ammonium nitrate (7.38 mg/100 g). The lower content of vitamin C was found in varieties of lettuce with red leaves (3.06-3.68 mg/100 g) compared to varieties with green leaves (11.12-15.48 mg/100 g). The accumulation of nitrate in lettuce leaves directly depends on the variety and the applied fertilizer. The highest nitrate content was with ammonium nitrate (2355.6 mg/kg). The use of urea had a limiting role in the accumulation of nitrate in lettuce leaves (1561.3 mg/kg), as well as the use of ammonium sulphate (1968.5 mg/kg). The accumulation of nitrate in lettuce varieties with red leaves was higher comparing to varieties with green leaves.

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Author's contributions

Lj. B. Rakočević conducted the experiment, analysed the results, and drafted the manuscript. N. Pavlović provided the research design, participated in the research analysis and has drafted and revised the paper. J. Mladenović has participated in the chemical analysis and discussed the results. J. Zdravković was involved in data analysis and manuscript writing. I. Tošić revised and approved the manuscript. M. Marjanović has participated in the analysis and discussed the results. All authors read and approved the final manuscript.

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