RESEARCHES CONCERNING NITRATES AND NITRITES ACCUMULATION IN KOHLRABI IN THE WEST SIDE OF ROMANIA

Alina BULMAGĂ*, Aurel LĂZUREANU*, Ersilia ALEXA*, Monica NEGREA*

*Banat's University of Agriculture Sciences and Veterinary Medicine Timisoara, Romania
Corresponding author: Alina Bulmagă, Banat's University of Agriculture Sciences and Veterinary Medicine Timisoara, 119 Calea Aradului, 300645 Timisoara, Romania, tel.: 0040256277420, fax: 0040256277263, e-mail: alinaoliveiratop@yahoo.com

Abstract. In this study we followed the monitoring of nitrate and nitrite content in kohlrabi after the administration of organic and mineral fertilizers in different doses. Kohlrabi samples (GIGANT hybride) for the analysis have been taken from an experimental field established in Moşniţa, near Timişoara. In the experimental field to kohlrabi have been administrated three doses of synthetic fertilisers NPK $N_0P_0K_0$ $N_{30}P_{30}K_{30}$, (200 kg/ha NPK) $N_{60}P_{60}K_{60}$ (400 kg/ha NPK) and $N_{120}P_{60}K_{60}$ (400 kg/ha NPK + 180 kg/ha ammonium nitrogen) and one dose of organic fertilisers (organic compost - 30 t/ha).

The nitrate and nitrite content in kohlrabi was done from aqueous solution after spectrophotometric detection using the rapid tests AQUA MERCK and the Spectrophotometer SQ 118 at a wavelength of 515 and 525 nm for nitrate and nitrites. The experimental results show that the nitrate content, in samples taken from the Timiş County we registered values above maximum limit allowed for kohlrabi. The highest nitrate content (2180 ppm) was registered to variant V_4 where the used mineral fertiliser dose was $N_{120}P_{60}K_{60}$. This excessive accumulation appears because of the high nitrogen content N_{120} applied to this variant.

Keywords: kohlrabi, nitrate, nitrite, organic and mineral fertilizer.

INTRODUCTION

Kohlrabi comes from the wild specie *Brassica rupestris* that grows spontaneously in Mediteranne, the kohlrabi was cultivated from antiquity. Kohlrabi roots are consumed raw or cooked.

The amount of alimentary value is high in vitamins, minerals and sugars. With good storage capacity ensures the fresh food a long period of time [6].

Nitrate and nitrite are natural soil compounds, are proceed from organic, nitrogen substances mineralization. Nitrogen mineralization depends of the microorganisms that are in soil. A part of the nitrates and nitrites, is absorbed by the plants root and is a main material for protein synthesis and other compounds with nitrogen, and on the another way is trained by the surface waters on the one that travels the earth, until to the rivers, lakes or under waters. Natural, between nitrates and nitrites from soil, water and plants, it established a balance that can be broken by the intensive use in agriculture or horticulture of the natural organic or synthetic fertilisers. Their degradation products riches the soil and it can be accumulated in crops level for the consumers [7].

The analysis of the nitrates contribution provided by the solid nourishment (food), shows the weight of the ingeration of nitrates as a result of the vegetal origin food consumption (92%) and slightly because of the animal origin food (8%).

The highest values of the nitrates content is recorded at the vegetables with leaves and some root vegetables, in leaves and roots. The increased contribution of the nitrates brought by the vegetal consumption is owed to the use of those parts of the vegetables which are proved, accumulates the biggest nitrates part from the plant. The 71% contribution from the entire quantity of nitrates swallowed by the vegetables is owed to the consumption of the raw vegetables [1].

Vegetal species clasification in according with nitrates content lead to vegetable dividition in three classes:

- with high nitrates content (lettuce, spinach, red beet, radish, cabbage);
- with medium nitrates content (potato, caulliflower, patience, carrots, cabbage, kohlrabi);
- with low nitrates content (tomato, cucumber, pepper, melon, peace, fruits, cereals).

MATERIALS AND METHODS

In august 2008 was established an experimental field with kohlrabi in Moşniţa, near Timişoara, with the purpose to determinate the content of nitrogen compounds (nitrate, nitrite). The nitrate and nitrite content in kohlrabi, have been determinate for GIGANT hybride. The type of soil on which has been realized the experience was Black Chernozem with the following characteristics (Table 1):

Table 1. The soil characteristics of Black Chernozem from experimental field

Horizon (depth)	Soil characteristics							
	Clay	Sand (0.2-0.02 mm)	Sand (0.2-2 mm)	Dust (0.02-0.001 mm)	P (ppm)	pH in H_2O	Humic Matter (%)	N total (%)
Ap (0-25 cm)	41.1	29.2	0.5	29.2	28.8	6.45	4.09	0.136
Am (25-45 cm)	44.5	27.7	0.5	27.3	12.1	6.74	3.97	0.157

In the experimental field, to kohlrabi, hybride GIGANT, have been administrated three doses of synthetic fertilisers NPK and one dose of organic fertilisers:

V_1 – $N_0P_0K_0$

V_2 – $N_{30}P_{30}K_{30}$ fertilisers applied quantity 200kg/ha NPK fertilisers complex 16:16:16
V_3 – $N_{60}P_{60}K_{60}$ fertilisers applied quantity 400kg/ha NPK fertilisers complex 16:16:16
V_4 – $N_{120}P_{60}K_{60}$ fertilisers applied quantity 400kg/ha NPK fertilisers complex 16:16:16 + 180 kg/ha ammonium nitrogen (N_{60}).
V_5 – Organic compost - 30 t/ha

Experimental area for each variant: S = 8.40 m^2/ variant, each variant being realized in two repetition.

Fertilisers application has been made when the culture had 4-5 leafs. Taking samples and nitrate content determination have been realized after 8 weeks since the treatment and the analysis were made on turnip rooted.

Extraction and identification of nitrogen compounds: 50 g of sample it size degradation and it extract the nitrogen compounds with 500 ml water. The sample was filtrated and the filtrate was spectrophotometer analyzed. The nitrate and nitrite content in kohlrabi was done from aqueous solution after spectrophotometric detection using the rapid tests AQUA MERCK and the Spectrophotometer SQ 118 at a wavelength of 515 and 525 nm for nitrate and nitrites. Minimum detection limits according to work method are: 1 mg/l for nitrates and 0.02 mg/l for nitrites.

Calibration curve of nitrates and nitrites are shown in Figure 2. For each variant have been analyzed 3 repetitions, the obtained values being the mean of those 3 determinations. The regression coefficient and standard deviation for each curves was calculate and the value are: for nitrate (R=0.99546 SD= 0.01335) and for nitrite (R= 0.99404, SD=0.0733).

Figure 1. Nitrate and nitrite determination using AQUA MERCK rapid tests to Spectrophotometer SQ 118

RESULTS

The experimental results obtained about nitrate and nitrite levels in kohlrabi are given in Tables 2 & 3.

In our country the maximum limits of the nitrates and nitrites level in vegetable products are enacted with

Table 2. Nitrat content in kohlrabi in different variants

Variant	Weight of analysed sample (g)	Nitrat content in aquos solution (mg/l)	Nitrat content (ppm)
V_1	50	35.2	352
V_2	50	36.0	360
V_3	50	82.1	821
V_4	50	218	2180
V_5	50	74.6	746

Table 3. Nitrit content in kohlrabi in different variants

Variant	Weight of analysed sample (g)	Nitrit content in aquos solution (mg/l)	Nitrit content (ppm)
V_1	50	0.13	1.3
V_2	50	0.13	1.3
V_3	50	0.38	3.8
V_4	50	0.76	7.6
V_5	50	0.27	2.7

Order No. 293/640/2001-1/2002 regarding security and quality conditions for vegetables and fresh fruits destinated for human consumption, emitted by Order of Ministry of Agriculture, Alimentation and Forestry no. 293 from 2 august 2001, Order of Health and Family Ministry No. 640 from 19 september 2001, approved by Romanian National Authority For Consumers Protection in 3 january 2002 and published in Official Monitor No. 173 from 13 march 2002 (Table 4) [12-13]. The nitrite limits are not standardized, but should not exceed 1 - 2 ppm.

DISCUSSIONS

A comparative analysis of the action of the organic and mineral fertilizers with nitrogen on the nitrates content from the kohlrabi evidences at the same nitrogen doses, proves increased values at the nitrates level in the variants with mineral fertilization.

In the analyzed area, situated in a neighbourhood area to Timişoara, the mobile content from the soil is low, while the applied fertilizers quantities in the particular household are neglectable, so that the quantity of nitrogen accessible for plants is reduced, corresponds only to the nitrogen necessity of the plant for the achievement of protein-genesis, missing the nitric loading over the maximum admitted limit in the analyzed vegetable products [1].

In condition of excessive fertilization the nitrite content can increase above allowable limits. Experimental studies made on carrot culture to which have been administrated fertilisers with nitrogen, phosphorous and potassium in variant $N_{150}P_{90}K_{90}$ have showed the exceeding of the nitrites normal level. The nitrites content increasing was registered during the vegetation stages since 5-6 leaf stage of the plant until full maturity of the plant, to all the experimental variants to which has been administrated fertilisers [8].

Table 4. Maximum admitted limits of nitrates (NO_3 mg/kg) in some vegetables and fresh fruits for human consumption according with Order No. 293/640/2001-1/2002 of Romanian Ministry of Agriculture, Alimentation and Forestry

Product	Cultivated in	
	Field/ open area	Glasshouse
Pepper	150	400
Potato	300	N/A
Cucumber	200	400
Onion	80	N/A
Caulliflower	400	400
Kohlrabi	1500	N/A
Pumpkin	500	N/A
Carrots	400	N/A
Green lettuce	2000	3000
Red beet	2000	N/A
Spinach	2000	3000
Tomato	150	300
Cabbage	900	N/A
Apple	60	N/A
Water_melon	100	N/A
Grapes	60	N/A

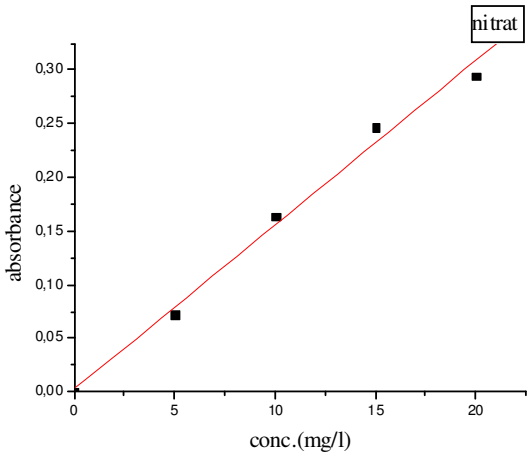

Figure 2. The calibration curve for nitrate identification

The highest nitrate content was registered to variant V_4 where the used mineral fertilizer dose was $N_{120}P_{60}K_{60}$. This excessive accumulation appears because of the high nitrogen content N_{120} applied to this variant. Researches made by another authors to carrot culture indicated nitrite content above maximum allowed limit using $N_{150}P_{90}K_{90}$ variant [8].

In the case of $N_{30}P_{30}K_{30}$ applied fertilizer (variant 2), the nitrate content is not different of the blind sample. Variant 3 (V_3 – $N_{60}P_{60}K_{60}$) lead to increasing of nitrate content in sample to 821 ppm without exceeding the standard maximum allowable limits, and in the case of variant 4 the nitrate content in sample was 2180 ppm, exceeding the maximum allowable limit of 1500 ppm.

The most recommended fertiliser dose applied to the kohlrabi culture is $N_{60}P_{60}K_{60}$, dose that didn't lead to excessive nitrate accumulation but lead to high production to unit of area.

The use of organic compost as fertiliser represents an optimal method to assure the necessary for the plant's development.

The nitrite content grows to those variants on which have been applied NPK inorganic fertilizer, accountable to blind sample, the increase being in direct proportion to applied nitrogen quantity. Nitrite content in kohlrabi should not exceed 1-2 ppm, but to variant V_4 - $N_{120}P_{60}K_{60}$ was registered 7.6 ppm, which is the highest value, followed by 3.8 ppm at V_3 and 2.7 ppm at V_5 variant. Previous studies regarding nitrate and nitrite content in cauliflower indicated lower values (between 200-300 ppm) for the same experimental variants [4]. This phenomenon appears because of the different capacity of the cauliflower and kohlrabi to accumulate nitrogen compounds [5].

The nitrate content which exists in plant at one time is the result of the balance between absorbed quantity and the one used in proteinogenesis. Any susceptible factor who interfere in metabolic chain, which assure the transformation of nitrate nitrogen in aminate nitrogen and protein nitrogen, can influence the free quantity of nitrates in plant [16].

The large interval of nitrate content in some vegetables (spinach, lettuce) is dependent of the nitrogen nutrition and climatic conditions. The excessive fertilisation, with doses that exceed the nitrogen needs of the plant in the maximum consumption period, lead to increasing of nitrates content in plant. Bruce and Nowatzki (2001) [2] observed that the nitrogen fertilisation increase the quantity of nitrates in vegetables and have been establish strategies and measures regarding correlation between nitrogen applied doses and nitrates content from lettuce, spinach and radish [3]. Studies realized in Bulgaria, to spinach culture [7] have shown that the nitrate content increase also in the case of low nitrogen applied doses (20 kg / ha N). Schuphan (1997) observed that fertilisation with quantities 4^{th} time higher than normal quantity of fertilisers lead to high values of nitrates in spinach, exceeding the allowable limits, but nitrites quantities remain lower [11].

Climatic conditions (temperature, rain, light intensity) determine the nitrate level in plants, as a result of conditions which influence the reduction reaction. From those the light intensity has an important role, because intervene as energy source in reduction process of nitrates in plant (nitrate – nitrite – aminoacid). So, in months (may – september) when the day light, respectively light intensity is near to maximum, permits obtaining of some vegetables with low nitrates content, because is favourize the reduction reaction, mean time in autumn - winter months grows the nitrates content [2].

The experience in the present study has been realized in unprotected conditions, in field, in maximum light intensity, factors which determine the reduction reactions of nitrate and does not accumulate in plants.

The nitrite quantity from vegetable species grows once with plant's maturity, as follow of nitrate decomposition in nitrite under the action of nitrate-reduction enzymes. The decomposition process of nitrite in ammonium ion, and of inorganic fraction in inorganic compounds with low molecular weight is deferred once with full maturity of plants, a possible cause being the small content of cooper and lead in kohlrabi [9].

The nitric compounds accumulation in plants is realized in different ways depending by the vegetation period and harvesting time, climatic conditions and most by the light intensity, lead to nitrate reduction in plants and the nitrate content diminish in harvested products [10].

REFERENCES

[1] Alexa, E., (2003): Contaminanţi în produsele vegetale, Eurobit Printing House, Timişoara, pp. 20-21.
[2] Alexa, E., (2008): Contaminanți în produse horticole si cerealiere, Solness Printing House, Timişoara, pp.12-15.
[3] Seelig, B., Nowatzki, J., (2001): Working to Avoid Nitrogen Contamination, AE-1218, pp. 45.
[4] Bulmagă, A., Lăzureanu, A., Alexa, E., Negrea, M., (2008): Study regarding nitrate and nitrite content in cauliflower, from agro-food markets in Timişoara. An. Univ. Oradea, Fasc. Biol., TOM XV: 22-23.
[5] Bulmagă, A., Lăzureanu, A., Alexa, E., Negrea, M., Cozma, A., (2008): The monitoring of nitrates and nitrites content in the cauliflower from the Timis county. The 15th Symp. on An. & Envir. Probl., SZAB, Szeged, Hungary, pp. 351.
[6] Ciofu, R., (2003): Tratat de legumicultură, Ceres Printing House, Timişoara, pp. 721-724.
[7] Gangolli, S.D., van den Brandt, P, Feron, V., Janzowsky, C., Koeman, J., Speijers, G., Speigelhalder, B., Walker, R., Winshnok, J., (1994): Assessment of nitrate, nitrite, and N-nitroso compounds. Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. Sect. 292: 1–38.
[8] Negrea, M., Lăzureanu, A., Alexa, E., Bulmaga, A., (2008): Researches concerning nitrates and nitrites accumulation in carrots, along of the vegetation stages, Fasc. Biol., TOM XV: 58-59.
[9] Rădulescu, H., (1999): Poluarea nitrică a alimentelor, Mirton Printing House, Timişoara, pp. 100-103.
[10] Santamaria, P., (2006): Review Nitrate in vegetables: toxicity, content, intake and EC regulation. J. Sci. Food Agric. 86: 10–17.
[11] Schuphan, I., Gaipl, S., Herlitz, E., Schreiner, J., Tietz, U., (1997): Kriterien zur Erkennung von Bodenkontaminationen bei der Testung von Bodenextrakten mit Hilfe biologischer Wirktests - Ermittlung der "Grundlevel-Antworten" unkontaminierter Böden. Wiss. Umw. (ISU) 2, pp. 107-111;
[12] ***(2002): Norms regarding security and quality conditions for vegetables and fresh fruits for human consumption emmited through Order of Agriculture, Alimentation and Forestry, Romania.
[13] ***(2002): Order of Health and Family No. 640 from 19 september 2001, approved by National Authority for Consumers Protection to 3 january 2002 and published in Romanian Official Monitor, no. 173, from 13 march 2002.