Evaluation of Nitrates Content of Some Vegetable Species Cultivated in Open Field, Using an Enzymatic Method

V. Ionescu*, M. Catana*, L. Catana*, M. Negoita*, E. Iorga*, A. Balea*, G. Lilios**, F. Burnichi*** and G. Campeanu

*Institute of Food Bioresources, 021102, Bucharest, Romanaia, Phone/Fax: 021 2109128, email: valentin.ionescu@bioresurse.ro, http://www.bioresurse.ro

**Ovidius University, 900527, Constanța, Romania, Phone: 0241 605050, Fax: 0214 543536 email: liliosgabriela@yahoo.com, http://www.pharmaovidius.ro

***Vegetable Research and Development Plant for Horticulture, 120024, Buzau, Romanaia, Phone: 0238 722593, Fax: 0238 722560, email: burnichi@yahoo.com, http://scdlbuzau.ro

Abstract: This paper presents the results of researches performed to establish the influence of fertilization treatments on nitrates content of some vegetable species, cultivated in the field: cabbage, carrots, bell peppers and onion. In the culture technology of these vegetables, the following fertilization variants were applied (fertilizer being ammonium nitrate, with 33% nitrogen): V1 = 0 kg nitrogen/ha; V2 = 100 kg nitrogen/ha; V3 = 200 kg nitrogen/ha; V4 = 400 kg nitrogen/ha; V5 = 800 kg nitrogen/ha. An enzymatic method was used to establish the accumulation potential of nitrates in vegetables cultivated in the field. In the case of the high fertilization level, cabbage had the highest nitrates content $(698.19 \text{ mg NO}_3^{-}/\text{kg})$, and bell peppers the lowest nitrates content $(61.70 \text{ mg NO}_3^{-}/\text{kg})$.

Keywords: nitrates, vegetables, enzymatic method, fertilization

1. Introduction

Nitrites and nitrates are natural components of the soil from nitrogen mineralization of organic substances of plant and animal origin. Nitrogen mineralization takes place primarily through existing micro-organisms in soil. In areas with temperate climate, this process is carried out with maximum intensity in hot season [1].

Achieving a sustainable soil fertility reintegration involves the mineral, plant and animal in its natural flow. In natural conditions, soil organic matter is maintained in terms of intake of animal and vegetable, devoid of life, which falls on the surface of the land mass of dead roots, soil microorganisms that die, all incorporated by biological cycles characteristic of biocenoses [4].

Fertilizer application is made on the basis of careful analysis for each crop separately and for each type of soil, taking into account that its uncontrolled use can pollute soil, groundwater and end products. In determining the dose of fertilizer will take account of soil nutrient content, the degree of water supply plant, pre-plant, the planned production level on cultivated plot [2].

Vegetables and fruits can accumulate nitrogen as nitrates and nitrites. Mineral nitrogen amount in vegetable and fruit tissues is higher in the case of species where nitrates reduction is made in leaves and when light intensity and temperature are lower [3].

In this paper are presented results of the performed researches, to establish the influence of fertilization

treatments on nitrates content of some vegetable species, cultivated in the field: cabbage, carrots, bell peppers and onion. In the culture technology of these vegetables, there were applied the following fertilization variants (fertilizer being ammonium nitrate with 33% nitrogen): V1 = 0 kg nitrogen/ha; V2 = 100 kg nitrogen/ha; V3 = 200 kg nitrogen/ha; V4 = 400 kg nitrogen/ha; V5 = 800 kg nitrogen/ha.

In order to establish the nitrates content of vegetables cultivated in the field, studied, an enzymatic method was used [4].

2. Experimental

Within the performed researches for determination of nitrates content of some vegetable species, cultivated in open field, to which there were applied different fertilization variants, there were used: Cabbage (*Buzoiană* variety), Carrots (*Nabuco* variety), Bell peppers (*Splendens* variety), Onion (*De Buzău* variety).

Samples were supplied by the Vegetable Research and Development Plant for Horticulture Buzau. In culture technology of these vegetables, there were applied the following fertilization variants (fertilizer being ammonium nitrate with 33% nitrogen): V1=0 kg nitrogen/ha; V2=100 kg nitrogen/ha; V3=200 kg nitrogen/ha; V4=400 kg nitrogen/ha; V5=800 kg nitrogen/ha. For each fertilization variant, there were realised 4 repetitions.

^{*****}University of Agronomical Sciences and Veterinary Medicine, 011464, Bucharest, Romania

For determination of the accumulation potential of nitrates in vegetables cultivated in open field, studied, it was used an enzymatic method. In this method, nitrate is reduced by the reduced nicotinamide adenine dinucleotide (NADPH), to nitrite, in presence of nitrate-reductase (NR):

$$NO_3^- + NADPH + H^+ \xrightarrow{NR} NO_2^- + NADP^+ + H_2O$$

Amount of oxidized NADPH is stoechiometrically equal with nitrate amount. Decreasing of NADPH amount is measured through absorbance at λ = 340 nm.

3. Results and Discussion

Within experimental variant V1 (without fertilizer adding), nitrates content of cabbage is in the range $79.52-84.08~mg~NO_3^-/kg$. The average value of the accumulation potential of nitrates in this case is $80.76~mg~NO_3^-/kg$.

When is applied fertilizer, nitrates content of cabbage increases once with increasing of fertilizer dose (ammonium nitrate with 33% nitrogen). Thus, in the case of fertilization variant V2 (100 kg nitrogen/ha) cabbage has a nitrates content in the range $136.75-138.08~mg~NO_3^-/kg$, and the average potential of nitrates accumulation is $137.38~mg~NO_3^-/kg$, with 41.21% much more than in the case of none fertilizer applied.

Within fertilization variant V3 (200 kg nitrogen/ha) cabbage has a nitrates content in the range $288.94-290.75~mg~NO_3^-/kg$), and the average potential of nitrates accumulation is $289.95~mg~NO_3^-/kg$, of 3.59 times higher, than in case of none fertilizer applied.

Application of a fertilizer dose of 400 kg nitrogen/ha (fertilization variant V4), it determines increasing of 5.51 times of the average potential of nitrates accumulation in cabbage cultivated in these conditions, in comparison with those non-fertilized. In the case of this fertilization variant cabbage has a nitrates content in the range $444.54 - 445.92 \text{ mg NO}_3^-/\text{kg}$, and the average potential of nitrates accumulation is $445.32 \text{ mg NO}_3^-/\text{kg}$.

Use in the culture technology of a fertilizer dose of 800 kg nitrogen/ha (fertilization variant V5), it determines increasing of 8.65 times of the average potential of nitrates accumulation in cabbage cultivated in these conditions, in comparison with non-fertilized cabbage. In the case of this fertilization variant cabbage has a nitrates content in the range $697.63-698.85~\text{mg NO}_3^-/\text{kg}$, and the average potential of nitrates accumulation is $698.19~\text{mg NO}_3^-/\text{kg}$.

According to the obtained results, cabbage is a vegetable species with an average potential of nitrates accumulation. Figure 1 presents the variation of the nitrates content of cabbage cultivated in field, depending on fertilization level.

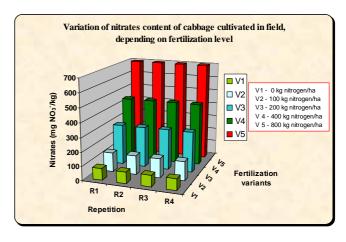


Figure 1. Variation of nitrates content of cabbage cultivated in field, depending on fertilization level

Within fertilization variant V1 (without fertilizer adding), nitrates content of carrots is in the range $90.18-91.88 \ mg \ NO_3^-/kg$. The average value of the accumulation potential of nitrates in this case is $90.85 \ mg \ NO_3^-/kg$.

When is applied fertilizer, nitrates content of carrots increases once with increasing of fertilizer dose (ammonium nitrate with 33% nitrogen). Thus, in the case of fertilization variant V2 (100 kg nitrogen/ha) carrots have a nitrates content in the range $159.55-161.93~mg~NO_3^-/kg$, and the average potential of nitrates accumulation is $160.98~mg~NO_3^-/kg$, with 77.19% much more than in case of none fertilizer applied.

Within fertilization variant V3 (200 kg nitrogen/ha) carrots have a nitrates content in the range $199.85-201.85\ mg\ NO_3^-/kg$, and the average potential of nitrates accumulation is $200.83\ mg\ NO_3^-/kg$, of 2.21 times higher, than in case of none fertilizer applied.

Application of a fertilizer dose of 400 kg nitrogen/ha (fertilization variant V4), it determines increasing of 2.87 times of the average potential of nitrates accumulation in carrots cultivated in these conditions, in comparison with those non-fertilized. In the case of this fertilization variant carrots have a nitrates content in the range $260.53-261.44~mg~NO_3^-/kg$, and the average potential of nitrates accumulation is $260.62~mg~NO_3^-/kg$.

Use in the culture technology of a fertilizer dose of 800 kg nitrogen/ha (fertilization variant V5), caused 4.3 times increasing of the average potential of nitrates accumulation in carrots cultivated in these conditions, compared with non-fertilized carrots. In the case of this fertilization variant, carrots have a nitrates content in the range $389.72-391.50~\rm mg~NO_3^-/kg$, and the average potential of nitrates accumulation is $390.49~\rm mg~NO_3^-/kg$.

According to the obtained results it can be concluded that carrots are a vegetable species with an average accumulation potential of nitrates. Figure 2 presents the variation of nitrates content of carrots cultivated in field, depending on fertilization level.

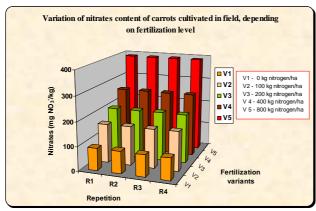


Figure 2. Variation of nitrates content of carrots cultivated in field, depending on fertilization level

Within fertilization variant V1 (without fertilizer adding), nitrates content of bell peppers is in the range $11.53-14.04~mg~NO_3^-/kg$. The average value of the accumulation potential of nitrates in this case is $12.63~mg~NO_3^-/kg$.

When is applied fertilizer, nitrates content of bell peppers increases once with increasing of fertilizer dose (ammonium nitrate with 33% nitrogen). Thus, in the case of fertilization variant V2 (100 kg nitrogen/ha) bell peppers have a nitrates content in the range $17.03-19.25\ mg\ NO_3^-/kg$, and the average potential of nitrates accumulation is $18.17\ mg\ NO_3^-/kg$, with 43.86% much more than in case of none fertilizer applied.

Within fertilization variant V3 (200 kg nitrogen/ha) bell peppers have a nitrates content in the range $27.10-29.35 \text{ mg NO}_3^-/\text{kg}$, and the average potential of nitrates accumulation is $28.08 \text{ mg NO}_3^-/\text{kg}$, of 2.22 times higher, than in case of none fertilizer applied.

Application of a fertilizer dose of 400 kg nitrogen/ha (fertilization variant V4), caused 3.22 times increasing of the average potential of nitrates accumulation in bell peppers cultivated in these conditions, in comparison with those non-fertilized. In the case of this fertilization variant bell peppers have a nitrates content in the range $39.67-41.55~mg~NO_3^-/kg$, and the average potential of nitrates accumulation is $40.67~mg~NO_3^-/kg$.

Use in the culture technology of a fertilizer dose of 800 kg nitrogen/ha (fertilization variant V5), caused 4.88 times increasing of the average potential of nitrates accumulation in bell peppers cultivated in these conditions, in comparison with non-fertilized bell peppers. In the case of this fertilization variant, bell peppers have a nitrates content in the range $60.85-63.08\,\mathrm{mg\ NO_3^-/kg}$, and the average potential of nitrates accumulation is $61.70\,\mathrm{mg\ NO_3^-/kg}$.

According to the obtained results, bell peppers are vegetable species with low potential of nitrates accumulation. Figure 3 presents the variation of nitrates

content of bell peppers cultivated in field, depending on the fertilization level.

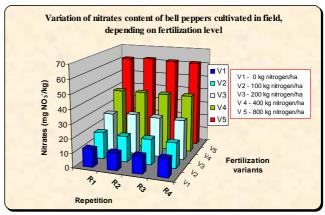


Figure 3. Variation of nitrates content of bell peppers cultivated in field, depending on fertilization level

Within fertilization variant V1 (without fertilizer adding), nitrates content of onion is in the range $28.35-31.15\ mg\ NO_3^-/kg$. The average value of the accumulation potential of nitrates in this case is $29.46\ mg\ NO_3^-/kg$.

When is applied fertilizer, nitrates content of onion increases once with increasing of fertilizer dose (ammonium nitrate with 33% nitrogen). Thus, in the case of fertilization variant V2 (100 kg nitrogen/ha) onion has a nitrates content in the range $49.21-51.45~mg~NO_3^-/kg$, and the average potential of nitrates accumulation is $50.27~mg~NO_3^-/kg$, of about 1.71~times~much~more~than~in~case~of~none~fertilizer~applied.

Within fertilization variant V3 (200 kg nitrogen/ha) onion has a nitrates content in the range $66.35-68.21\,\mathrm{mg\ NO_3^-/kg}$, and the average potential of nitrates accumulation is $67.32\,\mathrm{mg\ NO_3^-/kg}$, of about 2.28 times higher, than in case of none fertilizer applied.

Application of a fertilizer dose of 400 kg nitrogen/ha (fertilization variant V4), caused 2.38 times increasing of the average potential of nitrates accumulation in onion cultivated in these conditions, in comparison with those non-fertilized. In the case of this fertilization variant onion has a nitrates content in the range $69.20-71.35~\text{mg NO}_3^-/\text{kg}$, and the average potential of nitrates accumulation is $70.21~\text{mg NO}_3^-/\text{kg}$.

Use in the culture technology of a fertilizer dose of 800 kg nitrogen/ha (fertilization variant V5), caused 2.78 times increasing of the average potential of nitrates accumulation in onion cultivated in these conditions, in comparison with non-fertilized onion. In the case of this fertilization variant, onion has a nitrates content in the range $80.62-83.053 \ \text{mg NO}_3^{-}/\text{kg}$, and the average potential of nitrates accumulation is $81.81 \ \text{mg NO}_3^{-}/\text{kg}$.

According to the obtained results, onion is a vegetable species with low accumulation potential of nitrates. Figure

4 shows the variation of nitrates content of onion cultivated in field, depending on fertilization level.

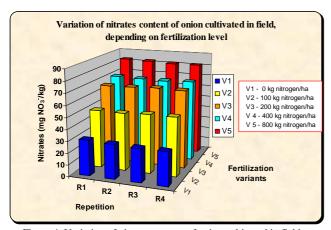


Figure 4. Variation of nitrates content of onion cultivated in field, depending on fertilization level

4. Conclusions

Cabbage samples obtained in field culture, through application of 5 fertilization variants, have a potential of nitrates accumulation in the range: $80.76~mg~NO_3^-/kg-698.19~mg~NO_3^-/kg$. The minimum value of nitrates content was found for non-fertilized cabbage, and the maximum value for fertilized cabbage with 800~kg~nitrogen/ha.

Carrot samples obtained in field culture, through application of 5 fertilization variants, have a potential of nitrates accumulation in the range: $90.85~mg~NO_3^-/kg-390.49~mg~NO_3^-/kg$. The minimum value of nitrates content was found for non-fertilized carrots, and the maximum value for fertilized carrots with 800~kg~nitrogen/ha.

Bell pepper samples obtained in field culture, through application of 5 fertilization variants, have a potential of nitrates accumulation in the range: $12.63 \ mg \ NO_3^-/kg - 61.70 \ mg \ NO_3^-/kg$. The minimum value of nitrates content was found for non-fertilized bell peppers, and the maximum value for fertilized bell peppers with 800 kg nitrogen/ha.

Onion samples obtained in field culture, through application of 5 fertilization variants, have a potential of nitrates accumulation in the range: $29.46~mg~NO_3^-/kg-81.81~mg~NO_3^-/kg$. The minimum value of nitrates content was found for non-fertilized onion, and the maximum value for fertilized onion with 800~kg nitrogen/ha.

ACKNOWLEDGEMENTS

The experiments were performed within the contract no. 51-050/18.09.2007, financed through Programme 4 "Partnerships in priority S&T Domains" 2007 – 2013 – National Centre for Projects Management.

REFERENCES

- 1. Bibicu M., Cercetari metodologice privind determinarea nitratilor si nitritilor din tesuturi vegetale si nivelului de acumulare in produsele horticole, Bucuresti, 1994.
- 2. Borlan Z., Dorneanu A., Nutritia minerala a plantelor in sisteme durabile de protectie vegetala si cu referire speciala la fertilizarea cu fosfor, Ed. Europa Nova, Bucuresti, 1999.
- 3. Gherghi A. et al., Biochimia si fiziologia legumelor si fructelor, Ed. Academiei Romane, București, 2001.
- 4. Luchian V., Legumicultura generala si speciala, Ed. Elisavaros, Bucuresti, 2007.
- 5.***, Protocol de lucru pentru metoda enzimatica UV de determinare a nitratilor din produse alimentare, R-Biopharm, **2008**.

Received: 06 April 2010 Accepted: 23 June 2010