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ORIGINAL RESEARCH PAPER

A COMPARISON OF NITRATE LEVEL IN SPINACH GROWN BOTH UNDER DIFFERENT DENSITIES IN AQUAPONIC SYSTEM AND UNDER NATURAL GROWTH CONDITIONS

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As bio-integrated systems that link both plants and fish culture, inside aquaponic systems, the processes of ammonia-oxidizing and nitrification in nitrite and nitrate are essential for growth and development of both fish and plants culture biomass. Plants with nearly the same nutritional requirements during their life cycle, like spinach in our case, are recommended to be grown under aquaponic conditions. Although nitrates concentrations up to 200 – 250mg/l are reported to be acceptable for fish growth, in the last years the toxic effect of *long term exposure* to high *nitrate* levels on fish and plants cultured biomass were highlighted. The main goal of the present study is to compare the nitrate level of spinach, grown in an aquaponic system, under three plant densities (V1 - 59 plants/m^2, V2 - 48plants/m^2 and V3 – 39 plants/m^2). The second objective is to compare the results obtained, in term of nitrate content, for spinach grown in the integrated rainbow trout – spinach aquaponic system with those of marketable spinach, grown under conventional condition, in the field. The experimental design consists in a recirculating aquaculture system with 12 growing units, mechanical and biological water treatment units and four aquaponic units. Fish were fed with two types of feed with 41% and 50% protein, using 3 different feeding regimes. The results show a higher nitrate level on spinach grown in aquaponic system, compared to the one derived from field culture. Differences were observed also among the three variants grown in aquaponic conditions, under different plant densities. As a conclusion, it can be said that considering the nitrate level, spinach grown in aquaponic system is marketable.

Keywords: nitrate level, aquaponic systems, plant densities, spinach, rainbow trout.

Introduction

The ability to produce crops intensively, with minimal to no impact to the environment is essential for human civilization to be able to meet future food demands without negatively impacting the environment (Licamele J., 2009). Aquaculture has evolved as the fastest growing food producing sector and developed as an important component in food security. Recirculating aquaculture systems (RAS) offer the great advantage of controlled culture conditions to optimize productivity, therewith to obtain high quality market products (Dediu L., 2012). Unfortunately, sometimes it has been demonstrated that RAS need an upgrade for increasing their profitability.

Therefore, to face the latest world requirements for sustainability, both industrial activities, intensive aquaculture and hydroponics, joined together as aquaponics.

One of the benefits of using an aquaponic system is that it can potentially reduce the amount of water used per kilogram of food produced up to 20-27%, compared to conventional agriculture systems (Chavez *et al.,* 2000). So, it can be said that in case of recirculating aquaculture systems, a method of improving effluent water quality characterized by high nutrient concentrations, especially nitrate, is to use hydroponic culture (Dediu L., 2011).

The second advantage is that greenhouse hydroponics production can produce from five to ten times more output compared to conventional agriculture (Resh, 2001; Hannan, 1998).

If in the case of recirculating aquaculture systems the main technological desideratum that must be accomplished is to ensure proper environmental conditions, that must correspond in a certain way to the ecophysiological particularities of the culture species (Cristea V., 2002), for aquaponic systems the perfect balance is provided by three aspects: plants species, fish culture species and environmental conditions.

Plants use ammonia and nitrates for growth (Marschner, 1995). Nitrate, that is taken up by the plant at better rates than ammonia nitrite, can be toxic to plants (Britto and Konzucker, 2002). Ammonia concentrations at elevated levels can inhibit nutrient uptake in plants by altering the ionic capacity of the water medium (Licamele J., 2009). The main part of the existing nitrogen is absorbed by the plant roots and serves as a starting material for synthesis of proteins and other nitrogen compounds.

Nitrates and nitrites are present both as undesirable contaminants and also as international additives in foodstuff (Fytianos and Zarogiannis, 1999). As a consequence, a great importance must be given when it comes to their concentration in different food products. It is well known that fresh leafy vegetables are major sources of dietary nitrate intake, fact generated by their nitrate accumulation capacity (Muramoto, 1999; Maynard, 1976; Lorenz, 1978). High nitrate concentration in vegetables is a worldwide problem. Comparing to nitrate, leafy vegetables nitrite content is quite low. Carrots, peas or potatoes seldom accumulate nitrates. On the other hand, broccoli, cabbage and especially lettuce and spinach have the tendency to accumulate nitrates (Firdevs Mor, 2010).

The aim of present study is to compare the roots - leaf nitrite and nitrate level of *Nores* spinach *(Spinacia oleracea)*, grown in an aquaponic system along with rainbow trout (*Onchorhynchus mykiss*), under three plant densities (V1 - 59 plants/m^2, V2 – 48 plants/m^2 and V3 – 39 plants/m^2). The second objective is to compare the results obtained for the spinach grown in the integrated rainbow trout – spinach aquaponic system to those of marketable spinach, grown under conventional condition, in the field.

Materials and methods

The present experiment took place between 20th february – 4th april 2013 and was carried out in the pilot recirculating system station of Aquaculture, Environmental Science and Engineering Department from Food Science Faculty- „Dunarea de Jos'' University of Galati. The recirculating system used for this experiment is equipped with 12 rectangular shape rearing units with a volume of 0.15m^3/unit, 2 rectangular sump units with a volume of 0.29 m^3/unit, one mechanical-quartz sand water conditioning unit, 1 biological trickling filtration unit, 1 sterilization UV filter, recirculating pumps, aeration pumps and sensors for monitoring water quality parameters.

The aquaponic modules consist in 4 rectangular glass made units (900x600x200mm), placed high above the recirculating system, on a metal support. A lighting system made of 4 fluorescent lamps, with red wavelength and a luminous power of 1080 lm was placed above the hydroponic units.

Regarding the water cycle inside the integrated system, it must be said that residual water from the rearing units passe first through mechanical filter and after that, through a recirculating pump, it goes to the biological filtration unit and then gravitationally to aquaponic modules, that flow out the treated water back to rearing units. The total volume of water from the integrated system is around the value of 2.5-2.7m^3. A water flow of approx. 0.8 l/s was set for the inlet of all 4 hydroponic units.

For the 44-day experiment, a total number of 228 rainbow trouts (*Oncorhynchus mykiss*) with an average initial weight of 111,77grams was used in parallel with spinach (*Spinacia oleracea*), *Nores* variety, at an age of 25 days. The seedlings were obtained at Natural Sciences Museum Complex, Galați - Botanical Garden. The total fish biomass from the recirculating aquaculture system, at the beginning of the experiment, was of 25.51kg.

Nores variety spinach was placed in the hydroponic units with the following stocking densities: (V1 - 59 plants/m^2, V2 - 48 plants/m^2 and V3 - 39 plants/m^2). Simultaneously, the fourth variant (Cv) consists in a number of 10 plants which were placed conventionally in soil, at Natural Sciences Museum Complex, Galați - Botanical Garden, to provide a comparation between conventional culture plants and aquaponic culture ones.

The support media of spinach, cultivated in the aquaponic system, consisted of polystyrene plates with holes for plastic special supports. The plants were placed in plastic supports and then, the supports were filled with a few hydroton balls to ensure their stability. The distance between plants equaled 15cm, both for aquaponic and conventional cultured ones. The maximum capacity of an aquaponic unit was 32 plants.

Fish were divided in six groups, in duplicate. Three of the groups were fed Clasic Extra 1 P (41% brute protein, 12% raw fats, 3% raw cellulose, 6.5% ash, 0.9% phosphorus, 14.2 MJ/kg digestible energy, 10000 UI vitamin A, 1250 UI vitamin D_3, 150 mg vitamin E, 75 mg vitamin C, 2.4% lysine, 0.75% methionine and 0.6% cystine) and the other three were fed Nutra PRO-MP-T (50% brute protein, 20% raw fats, 0.7% raw cellulose, 9% ash, 1.3% phosphorus, 19.7 MJ/kg digestible energy, 12000 UI vitamin A, 1800 UI vitamin D_3, 180 mg vitamin E and 500 mg vitamin C), as described by Hayward *et al.* (1997). A total amount of 6181.66 grams of Clasic Extra 1 P feed and 5789.77 Nutra PRO-MP-T was adminitrated during all 44 experimental days, in duplicate. A daily procentage of 10% water exchange was applied.

The technological water was analyzed in terms of temperature, pH, dissolved oxygen, conductivity, nitrates, nitrites and ammonium concentration.

The temperature and dissolved oxygen were daily monitored with a portable WTW ProfiLine Oxi 3205 Dissolved Oxygen Meter. The pH and conductivity were measured with WTW inoLab Multi 720 SET ph/Cond/Oxygen Meter and nitrogen compounds were determined by Spectroquant Nova 400 spectrophotometer, with Merk compatible kits.The luminous intensity was measured with TESTO 545 light meter.

The spinach cultured in the conventional system, at Natural Sciences Museum Complex Galați - Botanical Garden, was located in open field, without chemical treatments or fertilizers and the seedlings were obtained in the same way as those from the aquaponic system. The soil chemical analisys revealed a content of 0.4173%N. The determination of total nitrogen content was made by Kjeldahl method.

Chemical analyses concerning nitrite and nitrate levels in plants and plant roots were carried out on a number of 5 plants from each one of the variants, including conventionally cultured variant, using Griess method (STAS 9065 :2002).

The main goal of the present study is to compare, both at the beginning and at the end of the experiment, the nirate level of spinach, grown in an aquaponic system, under three plant densities. The second objective is to compare the results obtained for spinach grown in the integrated rainbow trout – spinach aquaponic system to those of spinach grown under conventional condition, in the field. The final results were statistically analysed using IBM SPSS Statistics 20.

Results and discussion

The values of water physico-chemical parameters, in the recirculating aquaculture system, registered values within the optimal range for both rainbow trout and spinach (Table1).

Table 1. Values of water physico-chemical parameters

Physico-chemical parameter	**Average value**
Temperature (^{0}C)	16.97±0.69
pH	6.78±0.27
Dissolved oxygen (mg/l)	7.24±0.49
Electrical conductivity (µS/cm)	1019.33±66.99
NH_4	0.21±0.1
NO_2	0.24±0.06
NO_3	96.42±8.01

By analyzing the values of main water quality parameters throughout the experimental period, it can be said that water temperature, pH and dissolved oxygen were maintained within optimal range for both rainbow trout and spinach (*Nores* variety) growth. Monitoring electrical conductivity will remove most of the guesswork in meeting the spinach nutritional needs (De Boer *et al*, 2012). It is well known that EC is an effective way to estimate the fertilizer content via salts. In our case, electrical conductivity registered slightly higher values, but similar to other authors as AL-Hafedh (2008). Ammonium and nitrites values were situated within the optimal range for the technological requirements for growing rainbow trout. Nitrate values were slightly higher, in agreement with Colt *et al,* (1981).

Regarding nitrite content in the consumable part of spinach from the initial batch, an average value of 0.61±0.074 mg/kg fresh weight is registered. Also, the nitrite content in V1 variant is 1.21±0.071 mg/kg fresh weight, 2.26±0.098 mg/kg fresh weight at V2, 2.24±0.085 mg/kg fresh weight at V3 and 0.93±0.11 mg/kg fresh weight at conventionally cultured spinach, Cv control variant (Figure1).

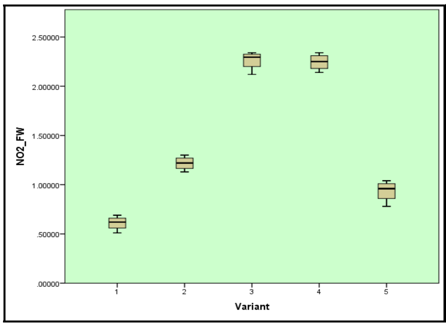

Figure 1 The level of nitrite from the consumable part of spinach, *Nores* variety (mg/kg fresh substance)*

*1-Initial (i); 2 –V1; 3 – V2; 4 - V3; 5 - Cv.

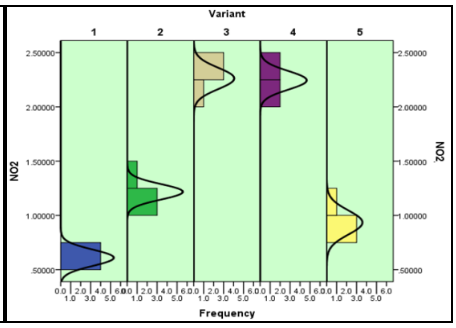

Figure 2 Distribution histogram of nitrite values from the consumable part of spinach, *Nores* variety (mg/kg fresh substance)

Normal data distribution (p>0.05) was found after applying Kolmogorov-Smirnov normality test. It can be seen (Figure 2) that the data distribution both in initial batch and in all experimental variants is mesokurtic with a little leptokurtic tendency. Also, by using two multiple comparison tests (Tukey and Duncan –

ANOVA), it was concluded that significant differences were found between all variants except for V3 and V4 between whom the difference was not significant ($p>0.05$; $p=0.99$). Tukey and Duncan tests divided the values in four homogeneous subsets: I; Cv; V1; V2+V3 (Figure 2).

Nitrite content from plant root samples had the following average values: 0.55±0.032 mg/kg fresh weight at V1, 0.35±0.044 mg/kg fresh weight at V2, 0.7±0.062 mg/kg fresh weight at V3 and 0.53±0.073 mg/kg fresh weight at Cv (Figure 3).

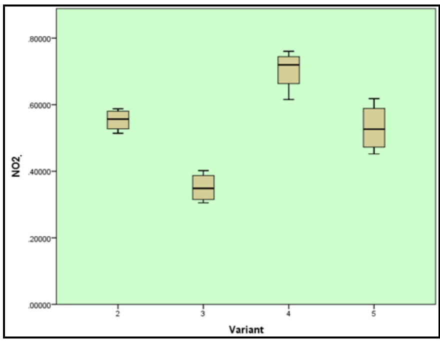

Figure 3 The level of nitrite from root samples of spinach, *Nores* variety (mg/kg fresh substance).*

***1-Initial (i) ; 2 –V1; 3 – V2; 4 - V3; 5 – Cv.**

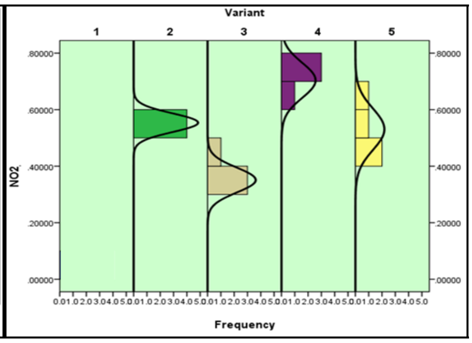

Figure 4 Distribution histogram of nitrite values from root samples of spinach, *Nores* variety (mg/kg fresh substance).

Kolmogorov-Smirnov normality test indicated a normal distribution of data ($p>0.05$). About the distribution, at Cv the mesokurtic distribution is clear comparing to V1, V2 and V3 where a little leptokurtic tendency was observed.

Using Tukey and Duncan post-hoc tests – ANOVA, it was concluded that significant differences ($p<0.05$) were found between all variants except for V2 and Cv between whom the difference was not significant ($p>0.05$). Tukey and Duncan tests divided the values in three homogeneous subsets: V2; V1+Cv; V3 (Figure 4).

The content of nitrate, that has been determined in the consumable part from the initial batch of spinach, before the beginning of the experiment (first group of values), registered an average value of 86.28±12.71 mg/kg fresh weight, with a minimum level of 70.4 mg/kg fresh weight and a maximum of 100.5 mg/kg fresh weight (Figure 5). At the end of the experiment, the average level of nitrate at V1 was 412.47±66.76 mg/kg fresh weight, in V2 variant was 540.29±2.4 mg/kg fresh weight and at V3 an average value of 716.31±59.98 mg/kg fresh weight. At the conventional growth method, the spinach cultured in the field (variant Cv) had a final average nitrate concentration of 157.08±45.34 mg/kg fresh weight (Figure 5).

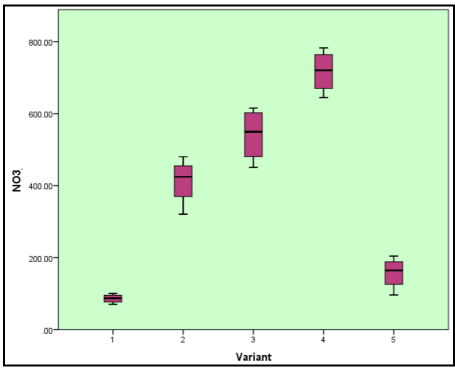

Figure 5: The level of nitrate from the consumable part of spinach, *Nores* variety (mg/kg fresh substance).*

*1-Initial (i); 2 –V1; 3 – V2; 4 - V3; 5 – Cv.**

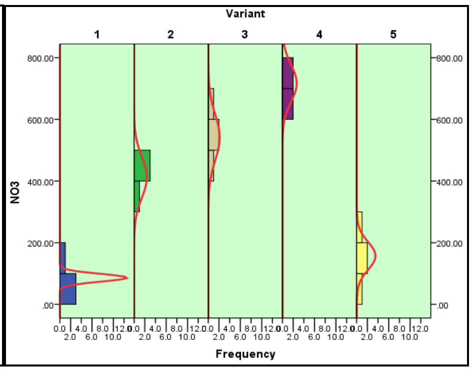

Figure 6: Distribution histogram of nitrate values from the consumable part of spinach, *Nores* variety (mg/kg fresh substance)

After applying Kolmogorov-Smirnov normality test on the obtained results, we found a normal distribution ($p>0.05$), fact which allowed us to apply the parametric tests. Regarding the data series distribution, by analyzing skewness and kurtosis, it can be said that in case of initial batch the mesokurtic distribution has a little leptokurtic tendency, a bit sharper than a normal distribution, more concentrated around the mean values, tilted to the right, having more extreme values to the left. At V1 and V2 the distribution is mesokurtic with slightly platikurtic influence, a bit flatter than a normal distribution, with the tendency of values scatting over a longer interval around the mean and also a little tilted to the left, with more extreme values to the right. The distribution at V3 and Cv is mesokurtic with slightly platikurtic influence and symmetrical around the mean (Figure 6).

To compare the nitrate level of all four variants and also of the initial batch, two multiple comparison tests (Tukey and Duncan – ANOVA) were used. We can state that between consumable parts nitrate content of the initial batch and V1, V2, V3, Cv variants, significant differences were found ($p<0.05$; p=0; p=0; p=0; p=0.42). Also between the values registered at V1, V2, V3 significant differences were found ($p<0.05$; p=0.038; p=0.004; p=0). The difference between the aquaponic growth spinach variants (V1, V2, V3) and conventional cultured spinach (Cv) were also significant ($p<0.05$). Tukey and Duncan post-hoc tests divided the values in four homogeneous subsets (i+Cv; V1, V2, V3).

The content of nitrate that has been determined in the root samples fresh weight, at the end of the experiment, had the following average values (Figure 7): 377±47.87 mg/kg fresh weight at V1, 418.68±61.51 mg/kg fresh weight at V2, 511.51±99.4 mg/kg fresh weight at V3 and 131.72±39.44 mg/kg fresh weight in case of Cv variant (Figure 7).

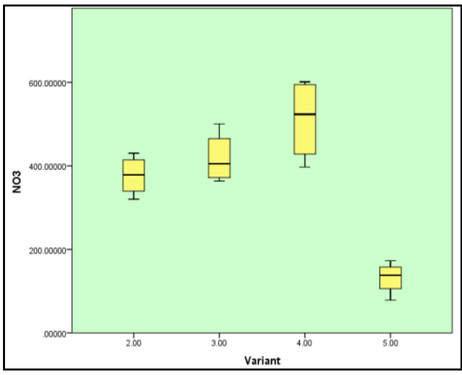

Figure 7: The level of nitrate from the root sample of spinach, *Nores* variety (mg/kg fresh substance). *

***1-Initial (i); 2 –V1; 3 – V2; 4 - V3; 5 – Cv.**

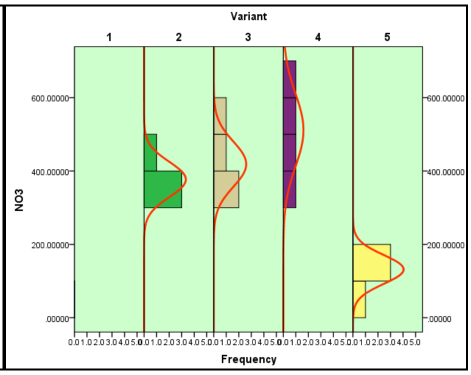

Figure 8: Distribution histogram of nitrite values from the root part of spinach, *Nores* variety (mg/kg fresh substance)

Kolmogorov-Smirnov normality test was processed on the obtained results and a normal distribution ($p>0.05$) was observed. Comments on data distribution, generated by skewness and kurtosis values, allow us to state that in case of V3 the distribution is mesokurtic, with platikurtic tendency, a bit flatter than a normal distribution, with the tendency of values scatting over a longer interval around the mean and also a little tilted to the left. At V1 and V2 variants the mezokurtic distribution has a bit leptokurtic influence, sharper than a normal distribution, more concentrated around the mean values. At Cv the distribution is mezokurtic, symmetric around the mean (Figure 8).

To compare the nitrate level from the roots of all four variants, two multiple comparison test (Tukey and Duncan – ANOVA) were used. As a conclusion, we can state that between the aquaponic growth spinach from V1, V2 and V3 variants and the conventional growth one (Cv), the differences were significant ($p<0.05$; $p=0.001$, $p=0$; $p=0$). Also between V1 and V3 root sample nitrate content, the differences are significant ($p<0.05$, $p=0.04$), while the differences between V1 - V2 and V2 - V3 were not significant ($p>0.05$, $p=0.81$, $p=0.24$).

Tukey test divided the values in two homogeneous subsets (Cv and V1+V2+V3) and Duncan test divided them in three homogeneous subsets (C; V1+V2; V2+V3)

The values of spinach nitrite and nitrate concentration mentioned in other scientific studies are presented in Table 2 and Table 3.

The nitrite and nitrate average levels of spinach, obtained from aquaponic production, are significantly higher than the one of conventional growth. However, the values do not exceed the acceptable upper limits of nitrates and nitrites from spinach (*Spinacia oleracea*) set up in Ordinance no 438/2002 – ordin MAPAM 438 – maximum allowance concentration of nitrates in spinach is 3000mgNO_3^-/kg fresh weight (Proca C., 2008). According to the same ordinance, values for children consumption are limited to 250 mgNO_3^-/kg fresh weight. In our case, only the conventionally grown spinach is within this range.

Table 2 Comparison of spinach nitrite mean value by various authors

Crt. no.	Author	Nitrite range	Nitrite mean value
1	Muresan C. *et al.*, 2012	1.54 - 8.09 mg/kg FW	-
2	Firdevs Mor *et al.*, 2010	-	7.189 mg/kg FW
3	Uwah *et al.*, 2009	-	44,85 ± 3,42 mg/kg FW
4	Aylin Ayaz *et al.*, 2007	ND – 12.11 mg/kg FW	2.31±2.63 mg/kg FW
5	Jaworska, 2005	0,09-0,77 mg/kg FW	-
6	Chung *et al.*, 2003	-	1 mg/kg FW
7	Jaworska G *et al.*, 1999	-	0.86 mg/kg FW 0.08g/kg DW
8	Petersen A. *et al.*, 1999	-	11 mg/kg FW
9	MAFF, 1998	-	3.8 mg/kg FW
10	Sumiko *et al.*, 1993	-	7 mg/kg FW

Table 3 Comparison of spinach nitrate mean value by various authors

Crt. no.	Author	Nitrate range	Nitrate mean value
1	Muresan C *et al.*, 2012	49.19 -399.5 mg/kg FW	-
2	Firdevs Mor *et al.*, 2010	-	1132 mg/kg FW
3	Centre for Food Safety Hong kong, 2010	3700 – 6300 mg/kg FW	4800 mg/kg FW
4	Centre for Food Safety Hong kong, 2010	-	3100 mg/kg FW
5	Korus A. *et al.*, 2009	2500 – 3000 mg/kg FW	-
6	EFSA, 2008	-	1066 mg/kg FW
7	Aylin Ayaz *et al.,* 2007	29.32 – 2478.26 mg/kg FW	1456.04±658,13 mg/kg FW
8	M. Shokrzadeh, 2007	24 – 457 mg/kg FW	223 mg/kg FW
9	Feng J. *et al.,* 2006	-	3177 mg/kg FW
10	Thomson B., 2004	-	824 mg/kg FW
11	Chung *et al.*, 2003	-	4259 mg/kg FW
12	Who, 2003	-	2824 mg/kg FW
13	Malmauret *et al.*, 2002	-	1135 mg/kg FW
14	Jaworska G. *et al.,* 2001	1000– 3472 mg/kg FW	-
15	Ministry of Agriculture, Fisheries and Food UK, 2001	25 - 4600 mg/kg FW	-
16	van der Schee *et al.*, 2000 and de Kreij C. *et al.*, 2000	30 - 6000 mg/kg FW	-
17	Muramoto J., 1999	419 - 1420 mg/kg FW	2400 mg/kg FW
18	Jaworska G *et al.*, 1999	-	1650mg/kg FW
19	Petersen A. *et al.*, 1999	-	1743 mg/kg FW
20	Santamaria *et al.,* 1999	-	1845 mg/kg FW
21	Ysart *et al.*, 1999	-	1900 mg/kg FW
22	MAFF, 1998	-	2470 mg/kg FW
23	Scharph *et al.*, 1991	900 - 5400 mg/kg FW	-
24	Sumiko *et al.*, 1993	-	3560 mg/kg FW
25	Dutt *et al.*, 1987	-	4570 mg/kg FW

The values of spinach nitrites obtained in this study are close to those of Jaworska., 2005, Chung *et al*, 2003 and Muresan C. *et al,* 2012 but generally, comparing to the values obtained by other authors, the levels are lower (Table 2). The nitrate levels are close to the levels reported by M. Shokrzadeh, 2007; Muresan C *et al,* 2012, but also lower than the ones reported from other authors (Table 3).

Conclusion

As a first conclusion of the present research, it can be stated that plant density influences nitrite and nitrate content of both consumable part and root of *Nores* variety spinach (*Spinacia oleracea*), grown in aquaponic conditions under an integrated recirculating systems with hydroponic units.

Also, as a second conclusion, this paper proves that *Nores* spinach variety (*Spinacia oleracea*), grown under aquaponic conditions with rainbow trout (*Oncorhynchus mykiss*), is marketable by meeting the food safety limits, having the nitrite and nitrate values within the allowable range.

So, as a final conclusion, an integrated rainbow trout – spinach aquaponic systems proves to be a suitable solution for feasible practice of aquaponics.

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