IMPACT OF NITROGEN DEFICIENCY ON BIOMASS PRODUCTION, MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF SWEET BASIL (*Ocimum basilicum* L.) PLANTS, CULTIVATED AEROPONICALLY

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Abstract

Sweet Basil was grown aeroponically under three levels of NO_3^- N (25, 50 and 160ppm) fertilization. Dry biomass production, plants height, roots length and number of leaves were dramatically decreased in nitrogen deficient plants. In addition, net photosynthesis and transpiration rates and stomatal conductance were also restricted, indicating that the primary metabolism was severely limited by low nitrogen availability. In contrast, nitrate accumulation in the leaves strongly decreased in relation with decreased nitrogen supply rates into nutrient solution. Also, total phenolics concentration significantly increased in N-starved plants indicating that biosynthesis of secondary plant metabolites is favored in nitrogen deficient plants.

Key words: *Ocimum basilicum* L., *nitrogen deficiency, growth, biochemical characteristics, total phenolics*

1. INTRODUCTION

Nitrogen (N) is one of the most important nutrients for plant growth, participating in many important functions of plant organisms (Nurzyńska-Wierdak et al. 2013). As a macro-element is absorbed by plants as nitrate (NO_3^-) ions or ammonium (NH_4^+) (Olfati, Khasmakhi-Sabet & Shabani 2012). Nitrate uptake and distribution in crops is of major importance with respect to both environmental concerns and the quality of crop products. A number of fundamental processes are regulated by N, resulting in profound changes in growth rate, net photosynthesis production, plant development, and yield. Many researches have been done in the past related to the effects of nitrogen fertilization on growth, yield, nitrate assimilation and plant secondary metabolites (Maršić & Osvald 2002; Prakasa Rao et al. 2007; Salahas et al. 2011; Nurzyńska-Wierdak et al. 2012). As it is well known nitrates are very important to the human diet with vegetables, constituting the most significant source (Amr & Hadidi 2001). According to Dennis et al. 2003, vegetables are giving the 75-80% of the total daily intake. Leafy vegetables, such us lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.) accumulate significantly high nitrate levels (Chang, Yang & Riskowski 2013).

Except from vegetables, herbs are also a great source of minerals, vitamins, phenolics and antioxidants, which are very important to human health, (Peyvast 2009). Phenolics are widely distributed secondary metabolites in the plant kingdom. Their accumulation in plants is a striking example of metabolic plasticity against biotic and abiotic stress factors, enabling plants to adapt to changing environments (Boudet 2007, Moreno et al. 2008). The interest in phenolic compounds has increased in the last decade, because of their presumed beneficial effects on human health due to their antioxidative and protective properties (Rice – Evans, Miller & Pagancga 1997). The biosynthesis and accumulation of phenolic compounds in plant tissues is strongly stimulated by nitrogen deficiency (Santsez et al. 2000, Sheible et al. 2004).

Basil (*Ocimum basilicum* L.) is one of the most popular culinary herbs, cultivated in many countries under natural and greenhouse conditions. It is marketed fresh, dried or frozen and it is used as ornamental, in cooking as a spice, in medical (Sgherri et al. 2010). Also it is cultivated for the production of essential oils (Tarchoune et al. 2013) which is used in food for aroma, in cosmetics and in medical (Lee et al. 2005).

In the present experiment, the method that used for the cultivation of Basil plants was aeroponics. Aeroponics is a non-substrate cultivation method, in which the roots grown in the air, misted with the nutrient solution (Zobel, Del Tredici & Torrey 1976). It is a system that allows access to roots without damage them (Hayden et al. 2004), enables the ability of controlling the culture conditions (Martin-Laurent et al. 1999) and optimizes the root aeration (Soffer & Burgeet 1988), resulting the increase of the roots growth (Zobel, Del Tredici & Torrey 1976). Previous researches (Burgess, McComb & Hardy 1998; Martin-Laurent et al. 1999; Chiipanthenga et al. 2012) have shown that this method have been used successful in many experiments.

The purpose of this work was to investigate the effect nitrogen starvation on morphological, physiological and biochemical parameters of Basil plants cultivated aeroponically.

2. METHODS AND MATERIALS

This study was conducted during Autumn-Winter 2013 in an automated glasshouse located at the former Technological Educational Institute of Messolonghi at Western Greece.

2.1. Plant growth

Sweet basil seed plants 10cm height were transplanted (after washing the roots in order to remove the soil) into the glasshouse fully automated aeroponic system. Plants were grown under natural temperature and daylight conditions. The closed recirculated aeroponic system consisted from 3 canals(x three replications). Each canal was made up of polystyrene of rectangular section with length 10m, width 0.67m and height 0.30m, with internal thin plastic layer, to allow reuse of the nutrient solution. The canals were covered with polystyrene panels with parallel holes for holding the plants. A high pressure irrigation system with two parallel pipes with sprayers was installed inside the structure. The roots of the plants were sprayed by the nutrient solution for 25sec every 5min. The system was fully electronically controlled.

2.2. Nutrient solution

The watering solution used for irrigation prepared with water at three different nitrogen treatment levels (25, 50 and 160ppm N), with all macro- and micronutrient concentrations held constant. The macronutrient composition (in mM), K, 6.5; Ca, 3; Mg, 0.9; $H_2PO_4^-$, 1.6 and micronutrient (in μM): Fe, 30; Mn, 5; Zn, 4; Cu, 0.75; B, 30; Mo, 0.53. The pH was adjusted to 5.6 by the use of H_2PO_4 and the electrical conductivity kept at 1.70 dS/m. The roots of the plants were sprayed by the nutrient solution for 25sec every 5min with a pressure of 5 atm. The system was fully electronically controlled.

2.3. Growth measurements

10 days after the beginning of the experiment and every 10 days the following parameters were measured: root and shoot fresh and dry weight, root length, shoot height and the number of leaves per plant.

2.4. Physiological parameters

The LCI Portable Photosynthesis System (ADC, BioScientific Ltd., England) was used for measuring photosynthesis rate, transpiration rate and stomatal conductance.By this system, CO_2 is measured by infrared gas analyzer and H_2O is recorded with two laser trimmed humidity sensors. During measurements the temperature was between 25°C to 32°C, under natural light (incident photon flux density on the leaf surface ≈ 1000 μmol m^{-2} s^{-1}) at the same daytime. Fully expanded leaves of the same physiological age of the plants were used.

2.5. Determination of total Chlorophyll Content

According to Shinano et al. (1996), three discs (0.9cm in diameter) from each leaf (first fully expanded leaf) were incubated with 3ml of dimethylsulphoxide (DMSO 99.5% or 14.8M; Sigma Chemical Co.) in a glass tube at 60^0C for 80min until the tissue became colorless. The absorbance at 665 and 648nm was measured with a spectrophotometer Shimadzu-UV-1601 (Shimadzu Corp., Japan). The total chlorophyll content was determined according to the following equations:

Chlorophyll a, Chla = 14,85xA665 −5,14xA648 (mg Chl a/ml)

Chlorophyll b, Chla = 25,4xA648 −7,36xA665 (mg Chl b/ml)

Total chlorophyll concentration (a+b), Chl(a+b) = Chla + Chlb (mg Chl/ml).

2.6. Determination of nitrate content

Nitrate concentration in crude extract of the leaves was measured with the portable spectrophotometer / reflektometer RQ-Flex 10 (Merck) and sample strips (Test Nitrates, 5-225 mg / l NO3-, 1.16971.0001, Merck). Fresh leaves of basil were washed with tap water and demonized water and after 1g was homogenized in an Ultra-Turret T 25 digital with 10ml of demonized water.

2.7. Determination of Total Phenolic Compounds

Samples of fresh leaves of basil (1g) were homogenized in an Ultra-Turrax T 25 digital in 10ml solution of Methanol: Deionized Water: Formic Acid in proportion 50ml:48.5ml:1.5ml respectively for 100ml solution (Mazza et al., 1999). The homogenate was centrifuged in a cooled centrifuge Centra MP4R (IEC, USA) at 10.000g for 10min at 4^oC and the supernatant was used for total phenolics determination. The absorbance of the supernatant was measured at 280nm in the Shimadzu-UV-1601 (Shimadzu Corp., Japan) spectrophotometer. The concentration of total phenolics compounds was determined using a Gallic acid standard curve and expressed as μgr GAE/ gr F.W.

2.8. Statistical data analysis

Values were compared by one-way ANOVA test and mean differences were determined using Duncan's test ($p<0.05$). The data analyzed using SPSS 17.0 program for Windows.

3. RESULTS

The dry biomass content, the root length, the shoot height and the number of leaves per plant were measured every 10 days,during growing season. Photosynthesis rate, transpiration rate, stomatal conductance, chlorophyll content, nitrate content and total phenolics content of roots and leaves were also determined in the same way.

3.1. Growth measurements

Nitrogen deficiency exerted significant effects on plant growth. All the morphological parameters affected from the different fertilization doses of N. Some of them affected from the beginning and some of them from the middle of the experiment with the lowest N application rate minimizing plant growth. According to Figures 1 and 2, dry shoot and root biomass production was negatively affected by eliminating N application rate. In both cases there were statistically significant differences between the three N fertilization rates (160, 50 and 25ppm). In both cases dry weight was found about 2 times lower at the lower N concentration rate into nutrient solution (25ppm), in comparison with the control (160ppm).

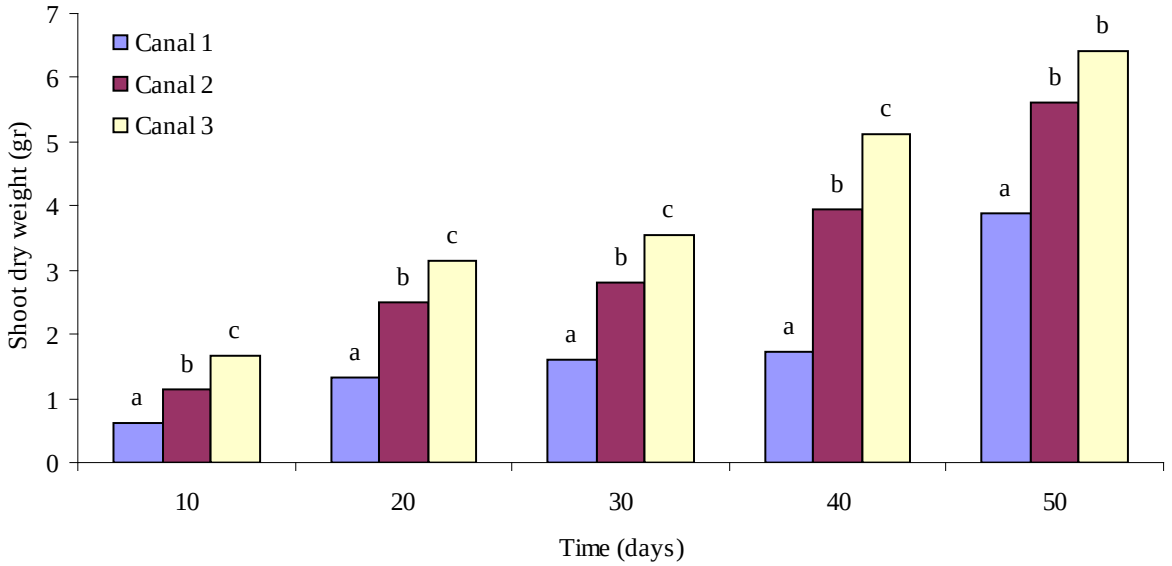

Fig. 1: Mean shoot dry weight of aeroponically cultivated Basil plants during cultivation season, in relation to the N-NO_3^- application rate. Canal 1: 25ppm N. Canal 2: 50ppm N. Canal 3: 160ppm N.

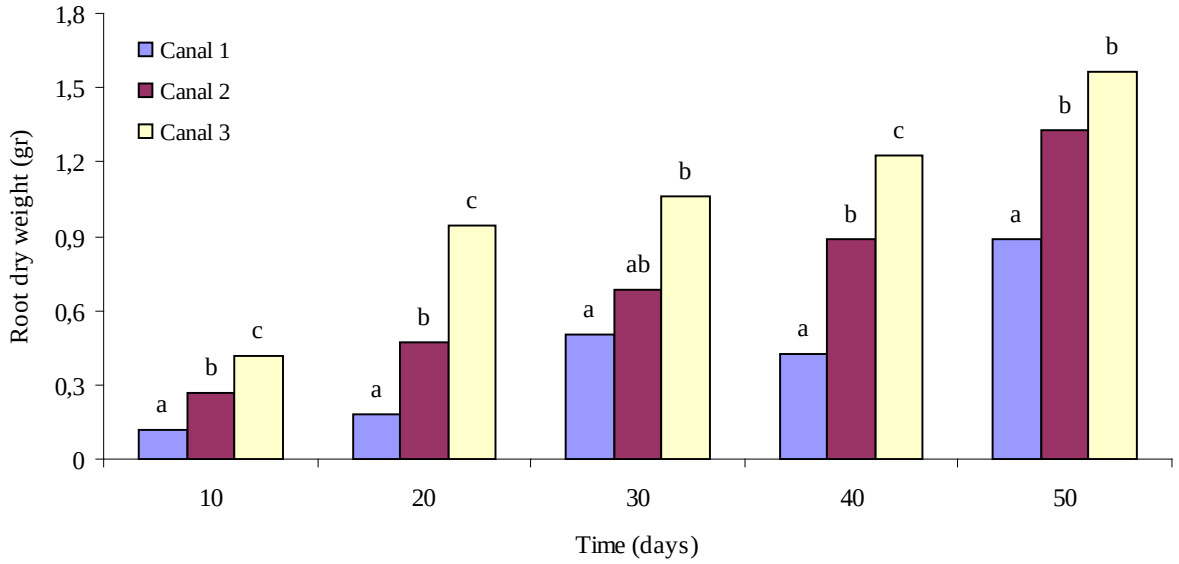

Fig. 2: Mean root dry weight of aeroponically cultivated Basil plants during cultivation season, in relation to the N-NO_3^- application rate. Canal 1: 25ppm N. Canal 2: 50ppm N. Canal 3: 160ppm N.

According to Fig. 3, root length was statistically decreased after 20 days of growing, as N fertilization rate was decreased. In the case of shoot height (Fig. 4), statistically the highest plants were observed at the highest N concentration rate into nutrient solution (160ppm). Also there were no statistical differences between 25 and 50ppm N up to the first month. Furthermore, as shown in Fig. 5 the number of the leaves per plant, 30 days after the beginning of the experiment exerted a significant decrease, particularly at the lowest N fertilization rate of 25ppm.

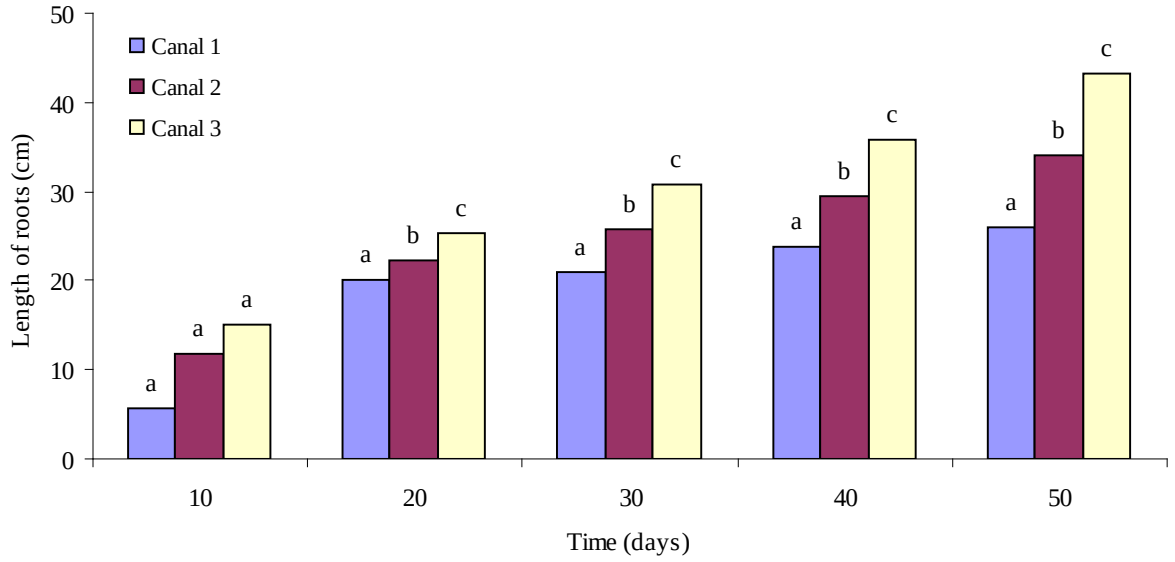

Fig. 3: Mean root length of aeroponically cultivated Basil plants during cultivation season, in relation to the N-NO_3^- application rate in the nutrient solution. Canal 1: 25ppm N. Canal 2: 50ppm N. Canal 3: 160ppm N.

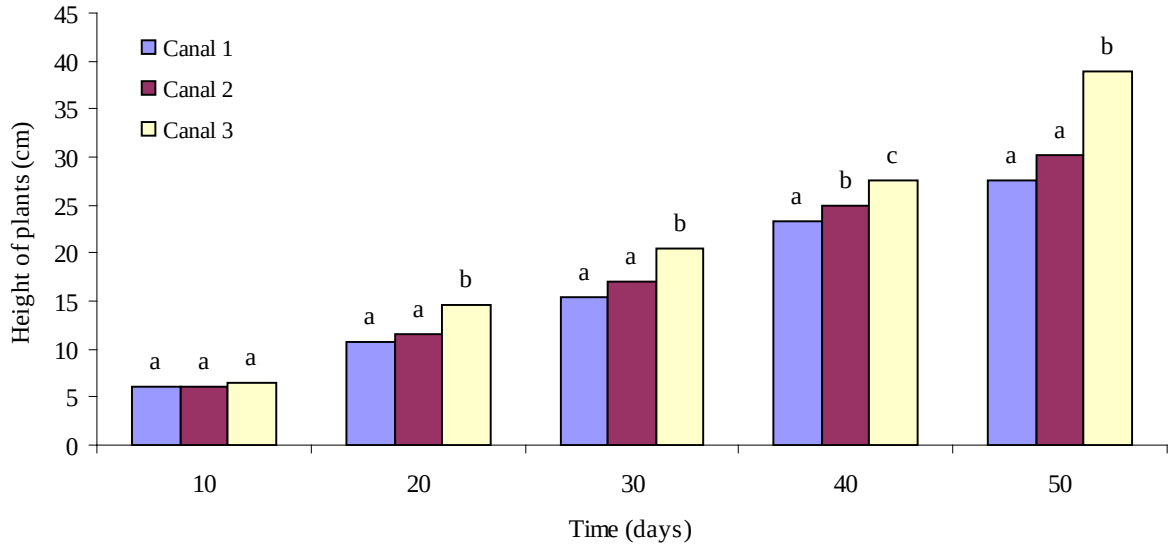

Fig.4: Mean shoot height of aeroponically cultivated Basil plants during cultivation season, in relation to the N-NO_3^- application rate. Canal 1: 25ppm N. Canal 2: 50ppm N. Canal 3: 160ppm N.

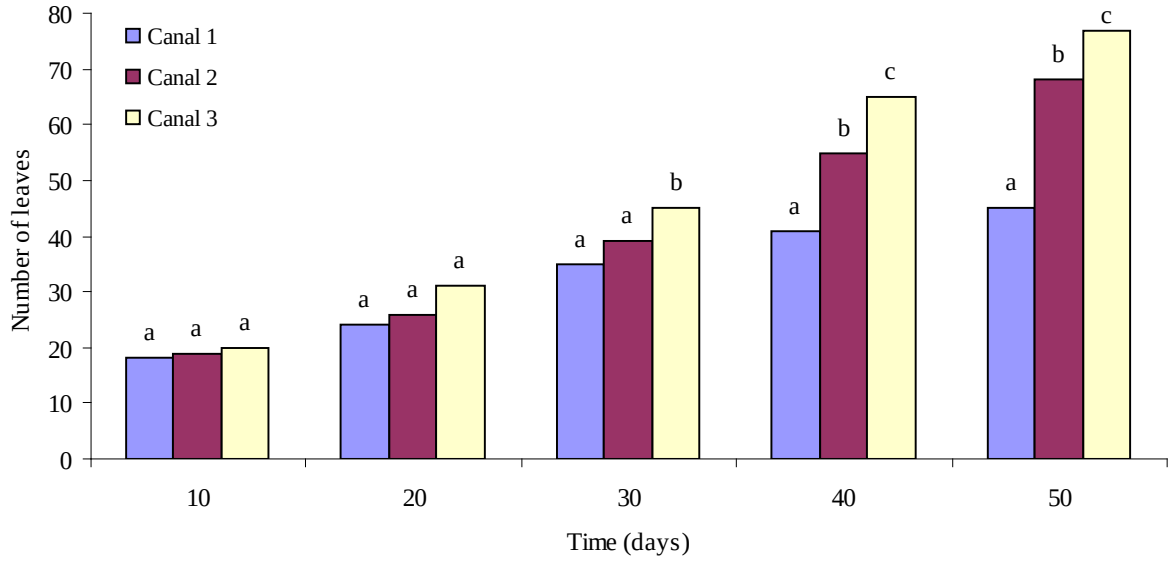

Fig. 5: Mean leaves number per plant of aeroponically cultivated Basil plants during cultivation season, in relation to the N-NO_3^- application rate. Canal 1: 25ppm N. Canal 2: 50ppm N. Canal 3: 160ppm N.

3.2. Total Chlorophyll Content and physiological parameters.

During the N-deficient treatment, the elimination of N- fertilization rate was significantly affected leaf total chlorophyll content. In particular, total chlorophyll concentration decreased from 12 mg/ gr DW to 7 mg/gr DW, with limiting N supply rates from 160 to 25 ppm N (Table 1). Furthermore, the gradual nitrogen deprivation in the growth medium from 160 to 25 ppm N, also caused a progressive decrease in the leaf physiological parameters as indicated by the measurements of net photosynthesis rate, transpiration rate and stomatal conductance (Table 1).

Treatments	Photosynthetic rate μmol m^{-2} S^{-1}	Transpiration rate mmol m^{-2} S^{-1}	Stomatal conductance CO_2 mol m^{-2} S^{-1}	Chlorophyll content mgr Chl/gr D.W.
25ppm N	3,33a	1,24a	0,13a	7b
50ppm N	7,48b	4,11b	0,24a	9b
160ppm N	13,27c	9,22c	0,46b	12a

Table 1: Impact of N-NO_3^- application rate on total chlorophyll content and physiological parameters in the leaves of aeroponically cultivated Basil plants during cultivation season.

3.3 Effects of nitrogen starvation on leaf nitrate content

The results indicate that nitrogen starvation had a significant negative effect on nitrate accumulation in plant leaves according to Figure 6. Statistically significant differences were determined by decreasing N application rates from 160 to 25ppm, indicating an analogous decrease in leaf nitrate content.

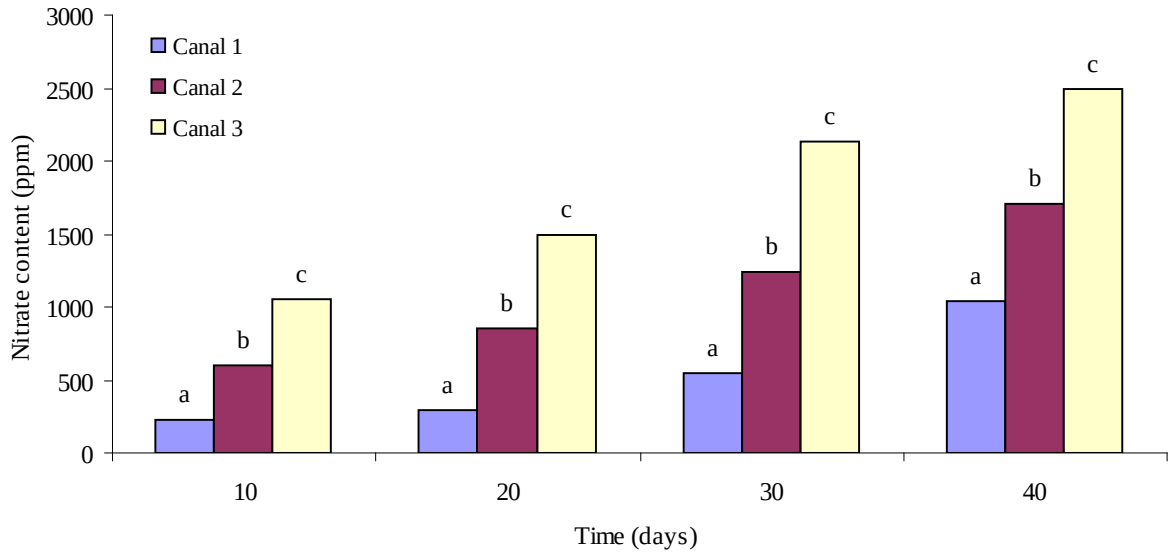

Fig. 6: Impact of N-NO_3^- application rate on nitrate content in the leaves of aeroponically cultivated Basil plants during cultivation season. Canal 1: 25ppm N. Canal 2: 50ppm N. Canal 3: 160ppm N.

3.4 Effect of nitrogen starvation on total phenolics content

The average total phenolics contents of Sweet basil grown with varying nitrogen fertilization levels are presented in Figure 7. The concentration of total phenolics in the leaves of the N-stressed plants, expressed as Gallic acid equivalents, significantly increased as N application rate was decreased from 160 to 25ppm. Total phenolic concentrations ranged from 1,66 mg GAE / g of fresh weight (F.W.) for plants treated with 160ppm nitrogen to 28,9 mg GAE / g F.W. for plants treated with 25ppm nitrogen. In particular, after 40 days of the beginning of the experiment, the concentration of total phenolics in the leaves of the plants grown at 25ppm N, rose by 296 % as compared with plants grown under unlimited N supply (160ppm N).

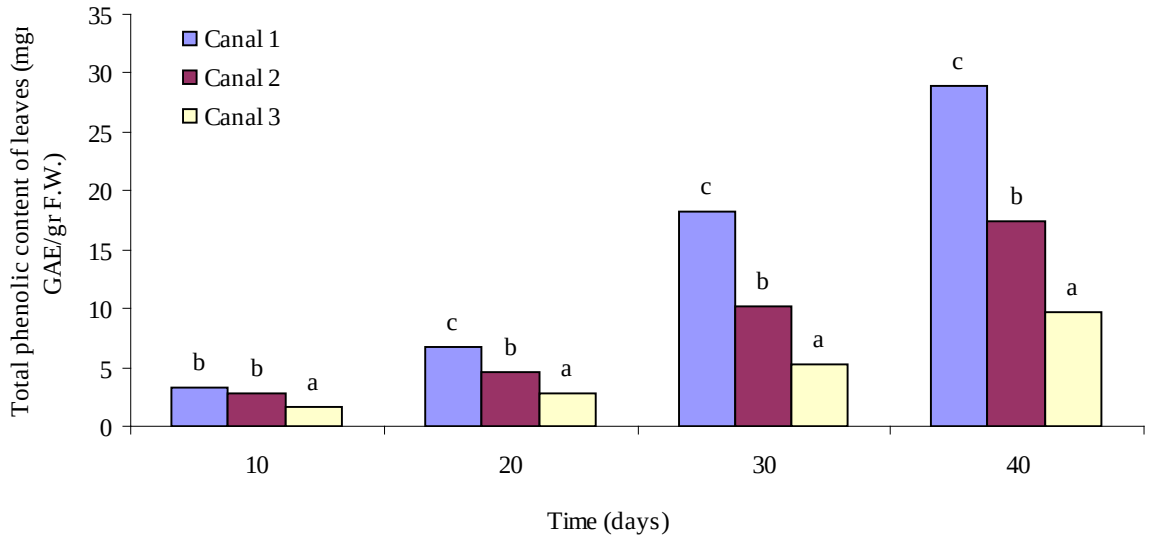

Fig. 7: Impact of N-NO_3^- application rate on total phenolics content in the leaves of aeroponically cultivated Basil plants. Canal 1: 25ppm N. Canal 2: 50ppm N. Canal 3: 160ppm N.

4. DISCUSSION

Nitrogen (N) is one of the most important macronutrients for plant growth and productivity and its availability affects many plant morphological, physiological and biochemical parameters (Steer & Hocking 1984). Plants require nitrogen in greatest amount, thus it is often the growth limiting macronutrient. From our results it is concluded that nitrogen deficiency significantly decreased growth parameters of sweet basil plants cultivated aeroponically, such as biomass accumulation, root length, shoot height and the number of leaves, in agreement with earlier findings with plants like salvia (*Salvia splendens* L.) (Kang & Van Iersel 2004), dill (*Anethum graveolens* L.), thyme (*Thymus vulgaris* L.) (Udagawa 1995) and lettuce (*Lactuca sativa* L.) (Maršić & Osvald 2002). According to Santos et al. 1998, Golcz, Politycka & Seidler-Łożykowska 2006 and Olfati, Khasmakhi-Sabet & Shabani 2012, nitrogen fertilization has been also shown to directly correlate with the growth, yield, and essential oil content of basil plants, in contrast with Jaćimović et al. (2010).

Furthermore from our results is revealed that nitrogen deficiency, compared to N replete controls, significantly decreases leaf CO_2 assimilation capacity of basil plant grown aeroponically. Net photosynthesis rate, transpiration rate, the stomatal conductance and the concentration of total chlorophylls, were strongly restricted by N deprivation rate, indicating that the primary metabolism is severely limited by low nitrogen availability, in accordance with previous observations (Lu & Zhang 2000, Duli Zhaoa et al. 2005). Antal et al. 2010, studding the acclimation of photosynthesis to nitrogen deficiency in *Phaseolus vulgaris*, have proved that N starvation induces a complex response, in which the plant down-regulates its photosynthetic capacity.

Previous results of our laboratory indicated that, in red beet plants grown hydroponically under N-deficiency stress, the allocation of N to secondary metabolic processes aimed at survival takes strong precedence over N utilization in growth processes. The elimination of N concentration into nutrient solution significantly reduced the total chlorophyll content and the leaf photosynthetic capacity. Those findings are in accordance with a previous work with maize plants (Ding et al. 2005).

Vegetables are generally considered the largest source of dietary nitrate, giving an average diet about 70-80% of total nitrate intake by humans (Hammes & Gierschner 1990). The significance of nitrate as human health hazard derives primarily from the fact that ingested nitrate is reduced to nitrite in the oral cavity at an average of about 4-8% (Gangolli et al. 1994). Nitrate accumulation in leafy vegetables primarily relates to the amount and form of N-fertilizer applied to the crop and is of grate importance for the nutritional quality of leafy vegetables (Bao-Ming Chen et al. 2004; Konstantopoulou et al. 2010). In the present work (and in agreement with the above findings), nitrate accumulation in the leaves of aeroponically grown basil plants, have shown a strong relation to nitrogen application rate into nutrient solution. Kiferle, Maggini & Pardossi 2013, who also investigated the influence of nitrogen (N) nutrition on biomass and RA production of sweet basil, suggested that the standard N concentration used in hydroponic culture (10.0 mol m^{-3} or higher) could be reduced considerably to 5.0 mol m^{-3}, with important implications from the environmental point of view. The need of farmers to obtain an adequate yield and a proper quality level, make it particularly difficult to take correct decisions about the dose, the timing and the typology of N fertilizer applications in order to achieve economic and environmental goals (Elia, Santamaria & Serio 1998).

Our results are in agreement with previous findings which show that the accumulation of phenolic compounds in plant tissues is often enhanced under conditions of restricted nitrogen nutrition (Mercure, Daoust & Samson 2004; Kováčik & Bačkor 2007). Lower levels of phenolic compounds in leaves and needles of plants grown under high N supply have also been reported for many other crop plants, including basil (Nguyen & Niemeyer 2008). Furthermore, our previous results with red beet plants grown hydroponically under N-deficiency stress indicated that the biosynthesis of secondary plant metabolites, such as total phenolics and betacyanins can be stimulated by N nutritional stress (Salahas et al, 2011). The manipulation of nitrogen fertilization levels may be an effective method to increase the concentration of polyphenolic compounds in red beet plants. Lower levels of phenolic compounds in leaves and needles of plants grown under high N supply have been reported for apple trees (Leser & Treutter 2005). Increased levels of most of the 11 phenolic acids detected in *Matricaria*

chamomilla L. leaf rosettes, subjected to nitrogen (N) deficiency, while different patterns of benzoic and cinnamic acid derivatives accumulation indicate that synthesis of these two groups of phenolics may be regulated independently (Kováčika et al. 2006, Kováčik & Bačkor 2007).

It is well documented in literature that N-deficiency stimulates the biosynthesis and accumulation of phenolic compounds, reallocating the available resources between plant growth and defense related processes (Tuomi, 1992; Herms & Mattson, 1992). There is a trade-off between primary and secondary plant metabolism in response to wide-ranging.

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