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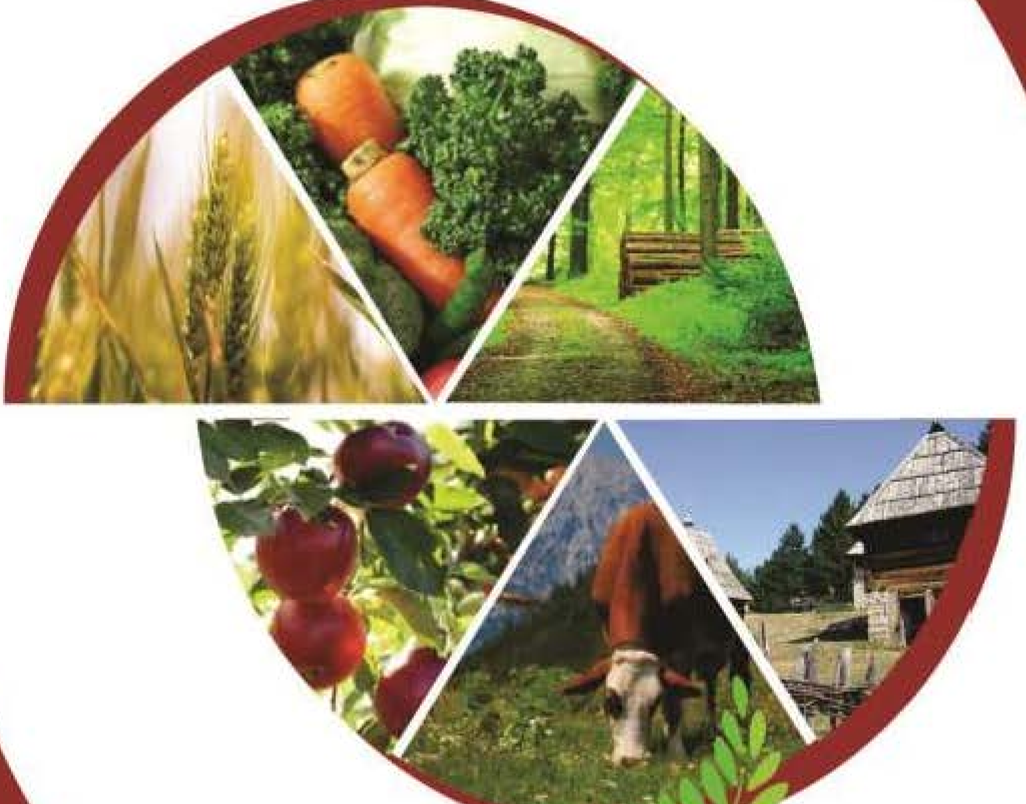

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THE EFFECT OF NUTRIENT SOLUTION COMPOSITION ON DEVELOPMENT OF *Cichorium spinosum* PLANTS

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Abstract

In the present study, the effect of nutrient solution composition on plant growth and quality of *Cichorium spinosum* L. was examined. Five fertilizer treatments were applied through irrigation water differing in the nitrate: ammonium nitrogen ratio of total nitrogen, namely (1) 100:0, (2) 75:25, (3) 50:50, (4) 25:75, 5) 0:100 NO_3:NH_4, while an extra treatment (6) with total nitrogen only in urea form was applied. All the treatments received the same amount of fertilizer units (20-20-20 mg L^{-1} of N, P and K, respectively). Plants were grown in 2 L pots containing peat (Klassman-Deilmann KTS2) and harvested three times during the growing period, and when they reached marketable size. At each harvest day, plant development was assessed (number, fresh and dry weight of leaves, and rosette diameter). The results suggest that nitrogen form has a significant effect on plant growth and yield of *C. spinosum* plants. In particular, fresh weight and number of leaves, and rosette diameter were significantly increased when ratio of nitrate: ammonium nitrogen was 75:25 or 0:100, especially in the 1st harvest, while in the 3rd harvest treatment 100:0 showed the best results. However, this did not affect total fresh weight and number of leaves which were higher for treatments 100:0 and 75:25, respectively. Therefore, higher ammonium nitrogen rates seem to be beneficial for plant development only during the early stages, while at later growth stages nitrate nitrogen has better results. In any case, ureic nitrogen is not suggested since it has severe effects on plant development, probably due to toxicity issues.

Keywords: *Ammonium nitrogen, Cichorium spinosum L., nitrate nitrogen, stamnagathi, urea*

Introduction

Cichorium spinosum L. is a wild edible chicory, which is self-grown in Crete and other regions of island and mainland of Greece. Rich in vitamins, minerals, fatty acids and antioxidants, with a distinct bitter flavor, it is also very popular abroad. Given the fact that it grows nearby coastal areas which suffer from high salinity, it is a well-adapted species under adverse conditions with considerably low cultivation needs (Chatzoulakis and Klapaki, 2006). As a fresh product, it has important nutritional value and achieves very high prices in the retail market. Therefore, it would be beneficial to study its cultivation for commercial purposes (Meliou at al., 2003; Zeghichi et al., 2003; Simopoulos, 2004).
Modern agricultural practices have rendered the application of fertilizers necessary for higher yields and better quality of final products. Nitrogen has a fundamental role in biosynthesis of acids, proteins, enzymes and chlorophyll of plants, rendering it an essential macronutrient (Barker et al., 1974). However, despite the positive effect of nitrogen fertilizers on yield and

quality, they can be toxic if supplied in irrational rates, especially in leafy vegetables which tend to accumulate nitrates with severe effects on human health (Hord et al., 2009). Moreover, apart from the overall nitrogen application rate, nitrogen form may affect the quality of the final product, especially phytonutrients content such as organic and fatty acids (Fontana et al., 2006; Szalai et al., 2010).

Although during the last few years farmers in many Mediterranean countries have started to commercially cultivate the species, scarce literature is available the nutrient requirements and the effect of fertilizers rates on yield. Therefore, the aim of the present study was to evaluate the effect of various ammonium and nitrate nitrogen rates on growth and development of *Cichorium spinosum* plants.

Materials and Methods

Plants were grown from seeds as previously described by Anesti et al. (2017). *Cichorium spinosum* L. seedlings were obtained from Vianame S.A. (Crete, Greece) at the stage of 3-4 leaves. Seeds were put in seed trays containing peat on 27/09/2016. Transplantation was carried out on 14/02/2016 and young seedlings were put in 2 L pots containing peat (Klassman-Deilmann KTS2). After transplantation, six fertilizer treatments were applied, namely (1) 100:0, (2) 75:25, (3) 50:50, (4) 25:75, 5) 0:100 NO_3:NH_4, while an extra treatment (6) with total nitrogen only in urea form was applied. One seedling was put in each pot, while each treatment consisted of 20 pots. Nutrient solution composition of each treatment is presented in Table 1. The various solutions were prepared by using appropriate amounts of the following fertilizers: a) ammonium nitrate (34.5% total nitrogen, with a ratio of 1:1 for NO_3-N: NH_4-N), b) potassium nitrate (13.5% of nitrate nitrogen and 46% of K_2O, c) magnesium nitrate (11% of nitrate nitrogen and 15% of MgO), d) monopotassium phosphate (52% of P_2O_5 and 34% of K_2O), e) ammonium sulphate (21% of ammonium nitrogen and 24% of 24 of SO_3), f) mono-ammonium phosphate (12% of ammonium nitrogen and 61% of P_2O_5), and g) urea (46% of ureic nitrogen).

Table 1. Nutrient solution composition for each treatment (mg L^{-1}).

	Treatments (NO_3^-:NH_4^+)					
Fertilizer type	100:0	75:25	50:50	25:75	0:100	Urea
Total nitrogen	200	200	200	200	200	200
NO_3^-	200	150	100	50	0	0
NH_4^+	0	50	100	150	200	0
Urea	0	0	0	0	0	200
K	200	200	200	200	200	200
P	200	200	200	200	200	200
Ca	0	0	0	0	0	0
Mg	178.3	136.3	0	0	0	0
S	0	0	0	136.4	252.8	24.0

Harvests was carried out on 22/03, 06/04 and 25/04/2016 for the 1st, 2nd and 3rd harvest, respectively. On each day of harvest, rosette diameter, number of leaves, fresh and dry weight were measured. For dry weight evaluation, samples of fresh leaves were oven dried at 72 °C to a constant weight (approximately for 48 hours). Water content was estimated as % percentage by subtracting dry weight from the initial fresh weight.

Experimental design was laid out according to Completely Randomised Design (n=20). Statistical analysis was conducted with the aid of JMP v 4.0.2 (SAS Institute Inc.) and Statgraphics 5.1.plus (Statistical Graphics Corporation). Data were evaluated by analysis of variance (ANOVA), whereas the means of values were compared by Tukey's HSD Test with $\alpha = 0.05$.

Results and Discussion

According to the results of Table 2, the ammonium fertilizer affects the growth and the quality of *Cichorium spinosum*. More specific, in the 1^{st} harvest fresh weight was higher in treatment 0:100, followed by treatment 75:25, whereas the treatment with ureic nitrogen had negative effect on yield. In contrast, Szalai et al. (2010) have reported a negative effect of high ammonium nitrate concentration in nutrient solution on fresh weight of purslane plants, which could be due to the shorter growth cycle of purslane comparing to *C. spinosum* plants that does not allow nitrification of ammonium nitrate to take place. Moreover, according to Marschner (1995) and Britto and Kronzucker (2002), the negative effects of ammonium nitrogen have been associated with changes of pH of growth medium and the toxicity of free NH_4^+. Considering that in our study no negative effects of ammonium nitrogen were observed in the early growth stages (1^{st} harvest), it could indicate a cumulative effect of NH_4^+ on growth media which is being established only at late growth stages and after the application of significant amounts of nutrient solution. In a previous study of Anesti et al. (2016), it has been suggested that high ammonium nitrogen rates (up to approximately 50% of total nitrogen) have a beneficial effect only when multiple harvest are applied which could also indicate the negative effect of accumulation of NH_4^+ in tissues of plants where no successive harvests are applied.

In the following harvests and especially for the 3rd harvest and for total fresh weight (Tables 3 to 5), the highest content of nitrates (100:0) in nutrient solution resulted in higher fresh weight. Ge (2002) and Wang et al. (2005) have also reported that high nitrate: ammonium nitrogen ratios resulted in higher plant yield of spinach, while Ulrich et al. (2017) have also suggested the beneficial effect of nitrates on fresh weight of lettuce plants. In the study of Ulrić et al (2017), it is also reported that high nitrate nitrogen rates increased leaf length and number of leaves, which also observed in the present study. This could be attributed to the fact that nitrogen in nitrate form is more easily available to plants than ammonium nitrogen and urea, as well as to the cumulative toxicity effects of ammonium nitrogen. This is more apparent after the first and second harvest where the temperature increase induces biosynthesis, therefore resulting in a significantly higher total fresh weight. Similar trends with fresh weight were observed for the number of leaves and rosette diameter in the 1^{st} and 3^{rd} harvest, with nutrient solution containing 100% of nitrate nitrogen having the better results, except for total number of leaves which was higher for treatment 75:25 (Table 2 and 4). However, this did not affect total fresh weight yield which was higher for treatment 100:0, as already mentioned. Dry weight did not show specific trends between the various treatments and harvests (Tables 2 to 5). In contrast, Wang et al. (2009) have reported an increase of dry weight in spinach leaves when ammonium nitrogen content increased.

In addition, the treatment where nitrogen applied in the form of ureic nitrogen had detrimental effects on plant development, probably due to toxicity effects which affected negatively all the measured parameters, except for dry weight of leaves.

Table 2**.** The effect of nitrate: ammonium nitrogen rates on development of *Cichorium spinosum* plants in the first harvest.

Treatment (NO_3^-:NH_4^+)	Fresh weight (g)	Rosette diameter (cm)	Number of leaves	Dry weight
100:0	13.9±2.6 bc	25.9±6.6 ab	12.9±3.4 c	9.1±1.3 ab
75:25	15.7±4.3 ab	29.1±8.7 a	17.4±6.1 a	8.8±1.0 b
50:50	13.2±1.7 cd	22.9±5.8 bc	14.5±4.6 abc	9.8±0.9 ab
25:75	13.5±1.6 c	24.1±3.8 bc	13.92.6 bc	10.6±0.2 a
0:100	16.7±3.2 a	26.0±5.5 ab	16.7±5.3 ab	9.4±0.8 ab
Urea	11.3±1.4 d	21.2±4.6 c	9.1±3.0 d	10.1±1.1 ab

*Means in columns followed by different letters without parenthesis, and means in columns followed by different letters in parenthesis are significantly different at p<0.05 by Tukey's HSD test.

Table 3. The effect of nitrate: ammonium nitrogen rates on development of *Cichorium spinosum* plants in the second harvest.

Treatment (NO_3^-:NH_4^+)	Fresh weight (g)	Rosette diameter (cm)	Number of leaves	Dry weight
100:0	12.9±2.2 a	25.3±3.7 a	7.7±4.3 c	6.7±0.9 b
75:25	12.1±3.1 a	22.8±5.4 b	7.7±2.4 c	6.4±0.7 b
50:50	10.7±0.9 b	20.6±3.7 b	8.1±3.0 bc	4.9±1.7 b
25:75	12.5±2.5 a	21.7±5.5 b	8.7±3.0 b	6.7±1.0 c
0:100	11.9±1.0 a	21.3±2.7 b	9.5±5.0 a	9.5±1.1 a
Urea	10.6±0.8 b	18.7±3.3 c	9.3±3.3 a	7.1±0.5 b

*Means in columns followed by different letters without parenthesis, and means in columns followed by different letters in parenthesis are significantly different at p<0.05 by Tukey's HSD test.

Table 4. The effect of nitrate: ammonium nitrogen rates on development of *Cichorium spinosum* plants in the third harvest.

Treatment (NO_3^-:NH_4^+)	Fresh weight (g)	Rosette diameter (cm)	Number of leaves	Dry weight
100:0	19.0±4.5 a	27.3±7.2 a	19.9±6.8 a	8.6±0.9 c
75:25	14.4±2.9 b	19.9±4.4 b	17.9±6.8 b	11.7±0.8 b
50:50	12.8±1.3 c	19.6±3.4 b	15.4±4.5 c	11.5±1.3 b
25:75	15.1±4.0 b	26.9±8.7 a	12±6.0 e	14.1±0.3 a
0:100	13.2±2.1 c	19.1±4.9 b	13.9±5.7 d	11.3±0.2 b
Urea	11.8±1.2 d	19.9±4.1 b	11.5±4.3 e	10.8±0.4 b

* Means in columns followed by different letters without parenthesis, and means in columns followed by different letters in parenthesis are significantly different at p<0.05 by Tukey's HSD test.

Table 5. The effect of nitrate: ammonium nitrogen rates on total fresh weight and number of leaves of *Cichorium spinosum*.

Treatment (NO_3^-:NH_4^+)	Total fresh weight (g)	Total number of leaves
100:0	45.8 a	40.5 b
75:25	42.2 b	43.0 a
50:50	36.7 c	38.0
25:75	41.1 b	34.6 c
0:100	41.8 b	40.1 b
Urea	33.7 d	29.9 d

* Means in columns followed by different letters without parenthesis, and means in columns followed by different letters in parenthesis are significantly different at $p<0.05$ by Tukey's HSD test.

Conclusions

The application of high nitrate rates (75:25 and 100:0 NO_3^-:NH_4^+) to *Cichorium spinosum* plants resulted in a significant increase of total fresh weight, mostly through the formation of more leaves. In addition, ammonium nitrogen (0:100) was beneficial only at the first harvest when plant requirements for nitrogen are lower comparing to the later stages when higher temperatures increase biosynthetic rates and therefore nitrogen uptake. Finally, ureic nitrogen had negative effect on fresh weight and total number of leaves, probably due to toxicity effects, therefore it cannot be proposed as a sole nitrogen source in nutrient solutions.

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