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EFFECT OF AMMONIUM FERTILIZER ON GROWTH AND QUALITY OF *Cichorium spinosum* PLANTS

Stavroula ANESTI[1], Konstantina FASOLI[1], Spyridon PETROPOULOS[1*], Georgia NTATSI[2], Vasilios ANTONIADIS[1], Isabel C.F.R. FERREIRA[3]

[1]University of Thessaly, Department of Agriculture, Crop Production and Rural Environment, Fytokou Street, 38446, Volos, Greece
[2]Agricultural University of Athens, Department of Crop Production, Iera Odos 75, 11855 Athens, Greece
[3]Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 1172, 5301-805 Bragança, Portugal
*Corresponding author: spetropoulos@uth.gr

Abstract

In the present study, the effect of ammonium fertilizer rate on plant growth and quality of *Cichorium spinosum* L. was examined. Five fertilizer treatments were applied regarding the ammonium nitrogen percentage of total nitrogen, namely (1) 14%, (2) 24%, (3) 34%, (4) 43%, and (5) 53% of total nitrogen applied in the form of ammonium nitrogen. All the treatments received the same total nitrogen rate. Seeds of *C. spinosum* were sown in seed trays containing peat, and young seedlings were transplanted in 2L pots containing peat and perlite in a ratio 1:1. Plants were harvested twice during growing period and when rosettes reached the marketable size. On the harvest days, plant features regarding plant development (the number of leaves, the fresh and dry weight of leaves, and rosette diameter), as well as quality mineral composition, SPAD index of leaves) were recorded. From the results it is suggested that fertilizer composition had a significant effect on plant growth and quality. In particular, fresh weight for both harvests, as well total fresh weight were higher in treatments 4 and 5, where 43 and 53% of the total nitrogen was applied as ammonium nitrogen. Dry weight did not differ significantly in the first harvest, while in the second harvest treatment 1 had the lowest dry weight. Diameter and number of leaves were the lowest in treatments 2 and 3, respectively, while significant differences were also observed between treatments regarding the quality features.

Keywords: *ammonium nitrogen, Cichorium spinosum L., nitrogen, stamnagathi.*

Introduction

Cichorium spinosum L., also known as "stamnagathi", is a native plant of the Mediterranean basin, which abounds in the coastal areas of the mainland as well as in many Greek islands, especially in Crete where it is well known for centuries and used as a vital component of the so-called Mediterranean diet (Melliou et al., 2003).
The fact that the species usually grows in coastal areas suggests a tolerance potential to high salinity and harsh conditions due to sea water intrusion in groundwater deposits (Chartzoulakis & Klapaki, 2000). Despite the fact that during the last few years farmers in Greece as well as in other Mediterranean countries have started to commercially cultivate the species with very promising results, scarce literature is available regarding the cultivation practices of *C. spinosum*, especially regarding the nutrient requirements and the effect of fertilizers on yield and quality of the final product.

Nitrogen is an essential macronutrient for plant growth, since it is a structural compound of chlorophyll, various enzymes, amino acids and proteins (Barker et al., 1974). As a fertilizer, it is one of the most used agrochemicals throughout the world, and is available in various forms, such as nitrate, ammonium and ureic nitrogen. Although the use of fertilizers has rapidly increased yield and farmers' income during the last decades, the irrational use of nitrogen fertilizers has many negative implications for the environment and the consumers' health. Excessive rates of nitrogen fertilizers can be a serious threat for human health, especially in the case of leafy vegetables that tend to accumulate nitrates (Hord et al., 2009). Nitrate accumulation in vegetables is dependent on various factors such genotype, growth conditions, and nitrogen form and application rate (Santamaria, 2006; Petropoulos et al., 2008). However, apart from nitrate content, nitrogen form may affect quality of the final product through the content of various phytonutrients such as organic and fatty acids (Fontana et al., 2006; Szalai et al., 2010).
The aim of the present study was to evaluate the effect of the ammonium nitrogen percentage of total applied nitrogen on both plant growth and quality of *C. spinosum*. For this purpose, five fertilizer treatments were applied, namely (1) 10%, (2) 20%, (3) 30%, (4) 40%, and (5) 50% of total nitrogen applied in the form of ammonium nitrogen.

Materials and Methods

Seeds of *Cichorium spinosum* L. (Asteraceae) were put in seed trays on October 10th 2015 containing peat and transplanted at the stage of 3 leaves (56 days after sowing) on December 5th, 2014 in 2 L pots containing peat (Klassman-Deilmann KTS2, 1.0 L) and perlite (1.0 L). After transplantation plants were fertilized with the same amount of nitrogen (300 mg L^{-1}) through the irrigation water with five fertilizer treatments which differed in their ammonium nitrogen content, namely (1) 14%, (2) 24%, (3) 34%, (4) 43%, and (5) 53% of total nitrogen applied in the form of ammonium nitrogen. In order to prepare the various solutions, the following fertilizers were used: a) 20-20-20 (N-P-K) with nitrogen consisting of urea (10%), NO_3-N (5.6%) and NH_4-N (4.4%), b) ammonium nitrate (34.5% total nitrogen, with a ratio of 1:1 for NO_3-N: NH_4-N), c) calcium nitrate (15.5% nitrogen, [NO_3-N (14.4%) and NH_4-N (1.1%)], and 26.5% CaO), d) urea (46% nitrogen in urea form), e) ammonium sulphate (21% of nitrogen in NH_4-N form, and 24% sulfur. Nutrient solution composition is presented in Table 1.
Plants were harvested twice during growing period and when rosettes reached the marketable size, while extra plants were harvested only once at the same day when the second harvest took place (uncut plants). The day of harvest, SPAD index of leaves was recorded, while after harvest, rosette diameter, number of leaves, and fresh and dry weight of 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. Dried samples were analysed for mineral composition. For mineral composition dried samples of leaves tissues were ground to powder, subjected to dry ashing and extracted with 1 N HCl to determine the mineral. Ca, Mg, Fe, Mn, and Zn content were determined by atomic absorption spectrophotometry (Perkin Elmer 1100B, Waltham, MA) and Na and K content by flame photometry (Sherwood Model 410, Cambridge, UK).
For plant growth, 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 for the main effects, whereas the means of values were compared by the LSD test and DMRT ($p = 0.05$). For chemical composition analyses, three samples were analysed for each treatment, whereas all of the assays were carried out in triplicate. The results were expressed as mean values and standard deviations (SD), and analysed using one-way analysis of variance

(ANOVA) followed by Tukey's HSD Test with α = 0.05. This analysis was carried out using SPSS v. 22.0 program (IBM Corp., Armonk, NY, USA).

Results and Discussion

From the results it is suggested that fertilizer composition had a significant effect on plant growth and quality features of *Cichorium spinosum* aerial parts. In particular, fresh weight for both harvests, as well total fresh weight for cut plants were higher in treatments 4 and 5, where 43 and 53% of the total nitrogen was applied as ammonium nitrogen, indicating that slow release of nitrogen is essential for higher yields. However, for uncut plants although total fresh weight was higher for treatment 4, it did not differ significantly from treatments 1 and 3 (Table 2). In addition, in any case total fresh weight was higher when plant were harvested twice comparing to plants that were harvested one time, while fresh weight for second harvest was significantly lower than that of the first harvest (Table 2). Similar results have been reported for other leafy vegetables, where multiple harvests increased significantly total yield comparing to single harvest (Kmiecik and Lisiewska, 1999; Csizinszky, 1999). This could be attributed to the fact that leafy vegetables that form rosettes are able to continue vegetative growth after harvest, as soon as the apex is not destroyed and growth conditions do not induce transition to flowering stage. 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.

Table 1. Nutrient solution composition expressed in ppm.

	Treatments				
Elements	1	2	3	4	5
N	299.95	300.13	300.40	300.01	299.97
NO3-N	108.87	138.86	148.81	131.00	110.99
NH4-N	41.20	71.19	101.14	129.07	159.05
Urea	149.87	90.07	50.44	39.93	29.92
Ca	75.22	75.22	75.22	23.93	23.93
S	0.00	0.00	22.86	45.71	102.85

Table 2. Fresh weight (g) of aerial parts of *Cichorium spinosum* in relation to fertilizer treatments and harvest stage.

	Fresh weight (g)					
Treatment	1st cut	2nd cut	LSD	Cut total	Uncut	LSD
1	29.3 bc(a)	21.4 bc(b)	4.7	50.7 b(a)	39.5 ab(b)	11.5
2	25.4 c(a)	16.4 d(b)	4.3	41.8 b(a)	29.0 b(b)	7.3
3	31.8 bc(a)	18.0 cd(b)	7.2	49.8 b(a)	34.3 ab(b)	12.4
4	41.7 a(a)	24.0 ab(b)	9.5	65.6 a(a)	44.0 a(b)	18.8
5	35.5 ab(a)	27.2 a(b)	7.4	62.7 a(a)	30.0 b(b)	12.5
LSD	8.5	4.7		11.8	11.4	

*Means in rows followed by different letters without parenthesis, and means in columns followed by different letters in parenthesis are significantly different at p<0.05 by LSD test.

Dry weight did not differ significantly in the first harvest, while treatment 1 and treatments 1 and 4 had the lowest dry weight in the second harvest and for uncut plants, respectively (Table 3).

Similar results have been reported by Wang et al. (2009) who also observed an increase in dry weight of spinach plants when nitrogen: ammonium nitrogen changed from 1:0 to 0:1. Additionally, uncut plants had higher dry weight than cut plants in almost every treatment (apart from treatment 4), which could be attributed to the consumption of more carbohydrate reserves for the regrowth of cut plants, comparing to the uncut plants which accumulated carbohydrates in larger amounts (Erice et al., 2011; Table 3).

Table 3. Dry weight (%) of aerial parts of *Cichorium spinosum* in relation to fertilizer treatments and harvest stage.

	Dry weight (%)					
Treatment	1st cut	2nd cut	LSD	Cut	Uncut	LSD
1	9.4±0.2	8.8±0.8b	1.3	8.8±0.8b(b)	10.9±0.2b(a)	1.3
2	9.5±0.4	10.2±0.1a	0.7	10.2±0.1a(b)	11.1±0.1ab(a)	0.3
3	8.5±0.4(b)	10.1±0.6a(a)	1.1	10.1±0.6a(b)	11.9±0.4a(a)	1.1
4	8.4±1.9	9.5±0.8ab	3.2	9.5±0.8ab	10.6±0.9b	2.0
5	8.7±0.4(b)	10.2±0.1a(a)	0.7	10.1±0.1a(b)	11.9±0.1a(a)	0.3
LSD	1.63	1.06		1.63	0.85	

*Means in rows followed by different letters without parenthesis, and means in columns followed by different letters in parenthesis are significantly different at p<0.05 by LSD test.

Diameter and number of leaves were the lowest in treatments 2 and 3 for both harvests, respectively, while for uncut plants treatment 4 resulted in higher rosette diameter, comparing to treatments 2 and 5 (Tables 4 and 5). Differences in rosette diameter were also observed between cut and uncut plants, only for treatments 4 and 5, indicating that higher amounts of ammonium nitrate have no beneficial effect on plant size when multiple harvests are applied (Table 4). In addition, ammonium nitrate has a positive effect on total number of leaves only when multiple harvests are applied, since slow release of nitrogen through nitrification process allows for better regrowth and leaf formation, comparing to plants where a single harvest is applied (Table 5).

Table 4. Rosette diameter (cm) of *Cichorium spinosum* in relation to fertilizer treatments and harvest stage.

	Rosette diameter (cm)					
Treatment	1st cut	2nd cut	LSD	Cut	Uncut	LSD
1	34.0 ab	35.3 ab	2.9	35.3 ab	40.1 ab	5.9
2	31.3 b	33.4 b	4.3	33.4 b	33.9 bc	4.3
3	39.3 a	37.4 ab	7.0	37.4 ab	38.9 ab	7.4
4	39.0 a	34.0 ab	5.3	34.0 ab(b)	42.9 a(a)	6.9
5	36.2 ab	37.9 a	4.8	37.9 a(a)	31.0 c(b)	5.0
LSD	5.3	4.4		4.4	7.5	

*Means in rows followed by different letters without parenthesis, and means in columns followed by different letters in parenthesis are significantly different at p<0.05 by LSD test.

Table 5. Number of leaves of *Cichorium spinosum* in relation to fertilizer treatments and harvest stage.

	Number of leaves					
Treatment	1st cut	2nd cut	LSD	Cut total	Uncut	LSD
1	34.8 a	26.7 a	10.7	61.6 a(a)	39.1(b)	19.3
2	28.0 ab(a)	20.1 ab(b)	4.4	48.1 b	40.1	9.5
3	24.4 b(a)	18.5 b(b)	5.6	42.9 b	35.4	12.3
4	29.0 ab(a)	22.1 ab(b)	5.6	51.1 ab(a)	35.7(b)	11.6
5	27.1 ab	23.7 ab	7.3	50.8 ab(a)	34.7(b)	10.9
LSD	7.1	6.8		11.2	8.1	

*Means in rows followed by different letters without parenthesis, and means in columns followed by different letters in parenthesis are significantly different at p<0.05 by LSD test.

Significant differences were also observed between treatments regarding the quality features. SPAD index was the highest when ammonium nitrogen constitutes 24%, 34% and 53% of total nitrogen in the first harvest, and 24% and 53% in the second harvest (Table 6). Moreover, SPAD index was higher in the second harvest and for treatments 2, 4 and 5, whereas the comparison of cut and uncut plants showed no specific trend being decreased, increased or unaffected by multiple harvests.

Table 6. SPAD index of *Cichorium spinosum* leaves in relation to fertilizer treatments and harvest stage.

	SPAD index					
Treatment	1st cut	2nd cut	LSD	Cut	Uncut	LSD
1	60.5 b(a)	51.4 c(b)	8.8	51.4 c(b)	100.7 a(a)	10.7
2	75.9 a(b)	84.6 a(a)	7.6	84.6 a(b)	94.9 a(a)	9.2
3	71.9 a(a)	76.8 b(a)	7.0	76.8 b(a)	80.1 b(a)	10.4
4	61.0 b(b)	71.9 b(a)	8.0	71.9 b(a)	54.4 c(b)	8.7
5	74.5 a(b)	86.9 a(a)	7.8	86.9 a(a)	96.6 a(a)	11.9
LSD	8.0	7.6		7.6	13.4	

*Means in rows followed by different letters without parenthesis, and means in columns followed by different letters in parenthesis are significantly different at p<0.05 by LSD test.

Mineral composition of the aerial parts was affected by fertilization treatments (ammonium: nitrate ratio) for most of the minerals evaluated in the present study (data not shown). Differences were also observed between the two harvests but only for specific minerals and fertilization treatments, while significant differences were detected in uncut plants (one harvest at the end of the growing season). In particular, K and Zn content was higher in the first harvest and for (3) and (4) fertilizer treatments, respectively, while Na, Mn and Fe content was higher in the second harvest and for (5), (3) and (1) fertilizer treatments, respectively (data not shown). In addition, cut and uncut plants differed in their mineral content, where in most cases cut plants had a higher content than uncut plants, especially in Fe content and for (3), (4) and (5) fertilizer treatments (data not shown). To our knowledge this is the first time that mineral composition of C. spinosum in relation to fertilization rates is studied; however mineral composition of wild or cultivated ecotypes has been already reported by (Petropoulos et al., 2016; Zeghichi et al., 2003; Zeghichi et al., 2003).

Conclusion

The application of high ammonium: nitrate rates (43% and 53% of total nitrogen) increases significantly total yield of C. spinosum only when multiple harvests are applied, mainly through the formation of more leaves that consequently result in higher total fresh comparing to a single harvest practice. The application of multiple harvests could increase the growing period and therefore allow for higher rates of ammonium transformation into nitrate nitrogen which is more readily available for plants, comparing to ammonium nitrogen. In addition, quality was not negatively affected by high ammonium: nitrate rates regarding SPAD index and mineral composition, indicating that multiple harvests combined with high ammonium: nitrate nitrogen could have a beneficial effect in terms of both quality and yield.

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