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# An Appraisal of Calcium Cyanamide as Alternative N Source for Spring-Summer and Fall Season Curly Endive Crops: Effects on Crop Performance, NUE and Functional Quality Components

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**Abstract:** A two-year study was conducted in both spring-summer and fall seasons to evaluate calcium cyanamide ($CaCN_2$) as an alternative nitrogen (N) source for curly endive (*Cichorium endivia* L. var. *crispum*) grown in a Mediterranean environment. Four types of N applications were administered: (i) pre-transplanting base application of 100 kg N ha$^{-1}$ corresponding to 100% of the supplied N ($100_{CC}$), (ii) pre-transplanting base application of 50 kg N ha$^{-1}$ corresponding to 50% of the supplied N ($50_{CC}$) complemented with 50 kg N ha$^{-1}$ as ammonium nitrate ($50_{AN}$) supplied through fertigation, (iii) standard application of 100 kg N ha$^{-1}$ as ammonium nitrate ($100_{AN}$) supplied entirely through fertigation, and (iv) a N-deprived control (0 kg N ha$^{-1}$) used as base reference to calculate the N use efficiency indices (NUE). Fall season increased head fresh weight, head height, stem diameter and plant visual quality, compared with the spring-summer season. The $CaCN_2$ and standard fertigation N applications were equally effective in increasing head fresh weight and other physical parameters such as, head height, stem diameter, visual quality, number of leaves and head dry matter when compared to the unfertilized control. However, in spring-summer season, $CaCN_2$, especially when applied straight at 100 kg N ha$^{-1}$, effectively increased ascorbic acid and total phenolic content, whereas, in fall season, an increase in TSS and ascorbic acid was recorded. In both, spring-summer and fall seasons, $CaCN_2$ significantly decreased N content and nitrogen accumulation ($N_{acc}$). Furthermore, $CaCN_2$ pre-transplant application improved NUE indices both in terms of N fertilizer recovery efficiency and in terms of physiological efficiency of applied N. Our results finally demonstrated that NUE indices increased in the fall season as compared to the spring-summer season.

**Keywords:** *Cichorium endivia* L. var. *crispum*; leafy green vegetables; cultivation season; nitrogen fertilizer; $CaCN_2$; functional properties

## 1. Introduction

Curly endive (*Cichorium endivia* L. var. *crispum*) is a leafy green vegetable belonging to the *Asteraceae* family, native to the eastern Mediterranean region and probably originated from the cross between *C. intybus* and *C. pumilum* [1,2]. Curly endive is an extensively grown vegetable in Europe and is praised for its typical crunchy texture and slightly bitter taste, rendering it suitable for direct consumption or as an ingredient of mixed ready-to-eat salads. Besides, curly endive is attractive due to its significant amount of nutritional and nutraceutical compounds such as minerals, ascorbic acid, phenolics, glucosinolates and sesquiterpene lactones [3–5]. Nitrogen above-optimal fertilization of vegetable crops represents a potential risk for human health. In particular, due to its accumulation in the edible part of leafy vegetables, $NO_3^-$ is considered an anti-nutrient [6,7] and therefore a cut back of its dietary consumption is suggested as a precautionary health care action. We assume that curly endive, although less cultivated than other leafy vegetables, is equally prone to $NO_3^-$ accumulation in the leaves when subjected to above-optimal nitrogen fertilization. Various authors reported that calcium cyanamide ($CaCN_2$) might be used as a soil fumigant for managing soil-borne diseases [8], pests and viruses in the soil [9], or as a tool to impede soil acidification, and expand yield and the quality of fruits [10,11]. However, $CaCN_2$ is also a well-recognized nitrogen fertilizer, particularly in the vegetable crop sector, and it may represent a valuable alternative to traditional soluble fertilizers. It may also improve the crop NUE [12] through a better modulation of N supply [13]. Several researches have demonstrated that nitrogen use efficiency (NUE) and $NO_3^-$ accumulation are affected by numerous interrelated factors such as fertilization rate and source, irrigation type, and growing season [14–20]. $CaCN_2$ application represents an easy, efficient and practical technique for leafy green vegetables fertilization. However, to our knowledge, there are no reports concerning the relationship between growing season and $CaCN_2$ fertilization doses for curly endive. Thus, the scope of our research was to assess the effects of various $CaCN_2$ pre-transplanting applications on yield, NUE, nutritional and functional traits of curly endive grown in spring-summer and fall seasons.

## 2. Materials and Methods

### 2.1. Plant Material, Growing Conditions and Treatments

The experiments were conducted in open field in two successive cropping seasons, spring-summer and autumn 2018 and repeated in 2019 at the Experimental Farm of the Department of Agricultural, Food and Forest Sciences of Palermo (SAAF), Sicily (Italy). Meteorological data were collected by the meteorological station of SAAF. Maximal and minimal daily temperature and rainfall in the course of the plant growth cycles were registered (Figures 1 and 2).

On 3 May and on 24 September 2018, curly endive (*Cichorium endivia* L., var. *crispum* Hegi) (var. Trusty, HM Clause, France) plug plants at the four-five true leaves stage were transplanted at a density of 10 plants $m^{-2}$. The soil of the experimental field was a Typic Rhodoxeralf soil made of sand, silt and clay with a percentage of 46.5, 22.3 and 31.2, respectively, and a pH of 7.2, highly rich in exchangeable $K_2O$, phosphorous, total nitrogen and organic matter with the following concentrations of 660 ppm, 68 ppm, 2% and 3.1%, respectively. Two doses of $CaCN_2$ at 100 kg N $ha^{-1}$ ($100_{CC}$) or at 50 kg N $ha^{-1}$ ($50_{CC}$) were applied by broadcasting on the whole plot surface and subsequently incorporating into the soil (10 cm depth) 505.0 kg $ha^{-1}$ or 252.5 kg $ha^{-1}$, respectively of granular $CaCN_2$ (19.8% of N) (Perlka; AlzChem, Trostberg, Germany). $CaCN_2$ was applied 15 days before transplant. The pre-transplant implementation treatment of 100 kg N $ha^{-1}$ ($100_{CC}$) (100% of the supplied nitrogen) was compared with the treatment including a pre-transplant implementation of 50 kg N $ha^{-1}$ ($50_{CC}$) (50% of the supplied nitrogen) by adding to the latter an implementation of 50 kg N $ha^{-1}$ ($50_{AN}$) supplied in the form of $NH_4NO_3$ (26% of N) by fertigation (192.3 kg $ha^{-1}$ $NH_4NO_3$). These two treatments were compared with a treatment including a conventional implementation of 100 kg N $ha^{-1}$ ($100_{AN}$) supplied in the form of $NH_4NO_3$ (26% of N) by fertigation (384.6 kg $ha^{-1}$ $NH_4NO_3$). Drip irrigation was applied to all plots as per standard grower practice and in order to avoid plant water stress. Two growing seasons (spring-summer vs. autumn) were combined with the four fertilization rates (Table 1) in a two factorial experimental design accounting for a total of eight treatments. Each treatment was made of three replicates with 10 plants per replicate and 240 plants overall. The experiment was repeated in 2019 in the same growing period.

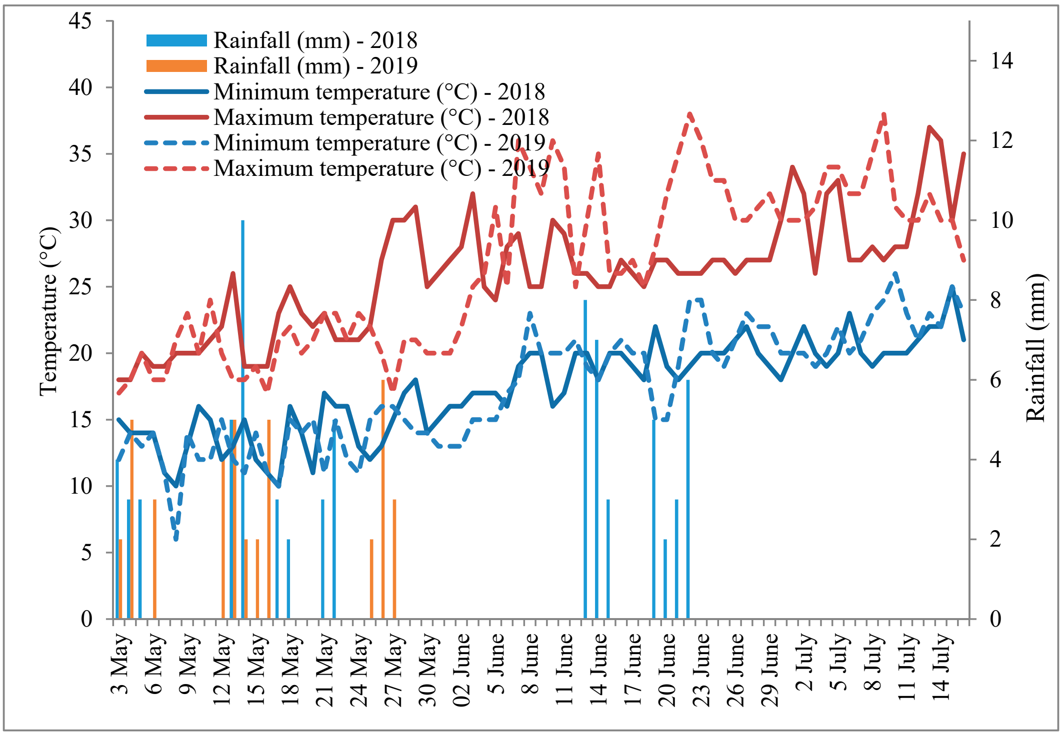


**Figure 1.** Daily minimum and maximum air temperature and rainfall at Marsala, Trapani province (longitude 12°26′ E, latitude 37°47′ N, altitude 37 m) during the spring-summer growing season (2018 and 2019).

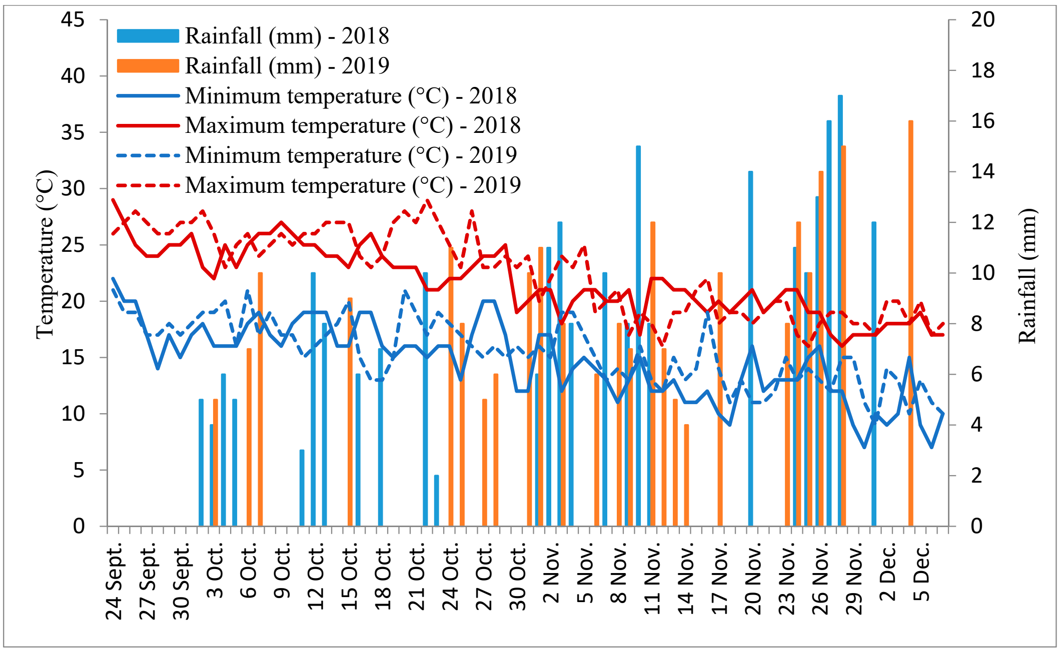


**Figure 2.** Daily minimum and maximum air temperature and rainfall at Marsala, Trapani province (longitude 12°26′ E, latitude 37°47′ N, altitude 37 m) during the fall growing season (2018 and 2019).

**Table 1.** Fertilization rates adopted for curly endive grown in spring-summer and fall seasons in a Mediterranean environment.

| Nitrogen Rate (kg ha$^{-1}$) | Nitrogen Source (kg ha$^{-1}$) | |
|---|---|---|
| | $CaCN_2$ (19.8% of N) | $NH_4NO_3$ (26% of N) |
| 0 | 0.0 | 0.0 |
| $100_{AN}$ | 0.0 | 384.6 |
| $50_{AN}$-$50_{CC}$ | 252.2 | 192.3 |
| $100_{CC}$ | 505.0 | 0.0 |

### *2.2. Growth, Yield and Plant Visual Quality*

Seventy-four days after transplanting curly endive plants were harvested. Afterwards, the following measurements were carried out for all the plants: head fresh weight and height, stem diameter and leaf number. At harvest, the visual quality of plants was also assessed based on a continuous score ranging from one to nine. Where one indicated a seriously damaged plant, three indicated a condition where the plant produced a usable but not saleable head, five indicated a fair condition where the head is at a marketability limit, seven indicated a good condition and nine to an excellent appearance. Five randomly selected plants from each replicate were sampled and placed in a thermo-ventilated oven (Memmert, Serie standard, Venice, Italy) to determine dry matter content. The first two days the oven was set at 80 °C and then at 105 °C until the samples reached constant dry weight.

### *2.3. Nutritional and Functional Traits*

The qualitative analysis samples were collected as stated by Sabatino et al. [5]. Thus, all samples were collected immediately after harvest. A sample of 200 g from each replicates was placed in a commercial juicer to extract its juice that was filtered later on. Then the content of soluble solids (SSC) was assessed by a digital refractometer (MTD-045nD, Three-In-161 One Enterprises Co. Ltd., New Taipei, Taiwan). Afterwards, through potentiometric titration with NaOH (0.1 M) up to a pH of 8.1, titratable acidity (TA) was evaluated. For this purpose, 15 mL of the extracted juice was used and the results were expressed in malic acid percentage equivalent [21]. A RQflex* 10 m reflectometer (Merck, Darmstadt, Germany) and Reflectoquant Ascorbic Acid Test Strips (Merck, Darmstadt, Germany) were used to measure ascorbic acid content. Distilled water was used to dissolve the leaf juice sample (1 g), where water was added and mixed until reaching a final volume of 10 mL. Then the test strips were immersed in the prepared samples and placed into the reflectometer. The obtained results were expressed as mg of ascorbic acid per kg fresh weight. Five grams of leaf sample extracted in methanol, were used to measure total phenolic content that was assayed quantitatively by A765 and expressed as mg of caffeic acid g$^{-1}$ fresh weight, according to the Folin–Ciocalteu method [22] with slight modifications [23]. Nitrogen (N) content was determined from the Kjeldahl method. Particularly, a sample rate was subjected to acid-catalyzed mineralization to convert the organic nitrogen into ammoniacal nitrogen. The ammoniacal nitrogen was then distilled in an alkaline pH. The ammonia created during this distillation was collected in a boric acid solution and determined via titrimetric dosage.

### *2.4. Nitrogen Accumulation and Nitrogen Efficiency Indices*

Total N accumulation ($N_{acc}$) was determined by multiplying head dry matter, expressed as kg ha-1, by its percentage content of N ($N_{acc}$ = head dry matter × Ncontent). Nitrogen efficiency indices were calculated immediately after harvest, as reported by Greenwood et al. [24]. The partial factor productivity of applied N ($PFP_N$) represents the harvested product (kg) per the amount of applied N (kg), where the calculation was done as follows:

$$PFP_N = \frac{YF}{NF} \quad (1)$$

where NF is the used N fertilizer (kg $ha^{-1}$), and $YF$ is the obtained crop yield (kg $ha^{-1}$) with the implementation of a noted NF rate (kg $ha^{-1}$).

The agronomic efficiency of applied N ($AE_N$) represents the increased yield (kg) per the amount of applied N (kg), where the calculation was done as follows:

$$AE_N = \frac{(YF - Y0)}{NF} \quad (2)$$

The apparent N fertilizer recovery efficiency ($REC_N$) by the crop represents the increased $N_{acc}$ (kg) per the amount of applied N (kg), where the calculation was done as follows:

$$REC_N = \frac{(AF - A0)}{NF} \quad (3)$$

The physiological efficiency of applied N ($PE_N$) represents the increased yield (kg) per the increased $N_{acc}$ (kg) from the applied fertilizer, where the calculation was done as follows:

$$PE_N = \frac{(YF - Y0)}{(AF - A0)} \quad (4)$$

where $YF$ is the crop yield (kg $ha^{-1}$) acquired with the implementation of a noted NF rate (kg $ha^{-1}$), $Y0$ is the crop yield acquired without the implementation of N fertilizer, $AF$ is plant total $N_{acc}$ (kg $ha^{-1}$) at maturity in the aerial biomass when N fertilization occur, and $A0$ is the corresponding plant total $N_{acc}$ (kg $ha^{-1}$) at maturity in the aerial biomass when no N fertilization occur.

### *2.5. Experimental Design and Statistical Analysis*

The SPSS software package version 14.0 (StatSoft, Inc., Chicago, IL, USA) was used to analyze the data, which were subjected to one-way analysis of variance. Tukey Honestly Significant Difference (HSD) test ($p < 0.05$) was used to separate the means when the nitrogen source was significant, and to separate the means of the treatment in case a significance was demonstrated for a particular measured characteristic. In order to evaluate the effect of the year, a preliminary two-way analysis of variance (nitrogen source × year) was performed.

## 3. Results

The experiment was repeated a second year using the same experimental scheme and obtaining similar results (Table S1). Therefore, data from 2018 are presented.

### *3.1. Crop Performance*

In spring-summer season, nitrogen-unfertilized plants had the lowest head fresh weight (Table 2). The latter was not significantly affected by the type of supplied nitrogen, as it ranged from 363.87 g in plants grown with $100_{AN}$ to 375.40 g in plants supplied with $50_{AN}$-$50_{CC}$. Data collected on head height, stem diameter and visual quality, number of leaves and head dry matter supported the trend established for the head fresh weight (Table 2). Although, plants cultivated in the fall season gave higher values in terms of head fresh weight, head height, stem diameter and visual quality, number of leaves and head dry matter, tendencies recorded in fall season were perfectly in accord with those recorded in the spring-summer season.

**Table 2.** Effect of nitrogen source on head fresh weight, head height, stem diameter and visual quality, number of leaves and head dry matter of curly endive grown in spring-summer and fall seasons.

| | Spring-Summer Season | | | | | | | | | | | | Fall Season | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Nitroge Source** | **Head Fresh Weight (g)** | | **Head Height (cm)** | | **Stem Diameter (mm)** | | **Visual Quality (1–9)** | | **Number of Leaves (No.)** | | **Head Dry Matter (g)** | | **Head Fresh Weight (g)** | | **Head Height (cm)** | | **Stem Diameter (mm)** | | **Visual Quality (1–9)** | | **Number of Leaves (No.)** | | **Head Dry Matter (g)** | |
| 0 | 270.70 | b | 19.73 | a | 19.00 | b | 4.33 | b | 46.33 | b | 17.13 | b | 330.8 | b | 23.23 | b | 21.7 | b | 5.00 | b | 59.00 | b | 25.13 | b |
| $100_{AN}$ | 363.87 | a | 22.27 | a | 22.33 | ab | 7.33 | a | 59.67 | a | 27.57 | a | 456.8 | a | 27.47 | a | 25.7 | a | 9.00 | a | 106.00 | a | 44.80 | a |
| $50_{AN}$-$50_{CC}$ | 375.40 | a | 22.00 | a | 22.67 | ab | 7.00 | a | 63.33 | a | 29.13 | a | 456.8 | a | 26.57 | a | 25.3 | a | 9.33 | a | 103.00 | a | 45.23 | a |
| $100_{CC}$ | 371.00 | a | 21.90 | a | 23.33 | a | 7.33 | a | 67.00 | a | 27.80 | a | 466.2 | a | 26.90 | a | 25.7 | a | 9.33 | a | 106.67 | a | 46.30 | a |
| Significance | *** | | NS | | * | | ** | | ** | | *** | | *** | | * | | ** | | *** | | *** | | *** | |

Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey Honestly Significant Difference (HSD) Test. The significance is designated by asterisks as follows: *** statistically significant differences at $p$–value below 0.001; ** statistically significant differences at $p$–value below 0.01; * statistically significant differences at $p$–value below 0.05; NS, not significant. 0, no nitrogen; $100_{AN}$, fertilized with ammonium nitrate at 100 kg N $ha^{-1}$; $50_{AN}$-$50_{CC}$, fertilized with ammonium nitrate at 50 kg N $ha^{-1}$ and with calcium cyanamide at 50 kg N $ha^{-1}$;$100_{CC}$ fertilized with calcium cyanamide at 100 kg N $ha^{-1}$.

### *3.2. Nutritional Traits, Functional Properties and N Accumulation*

In spring-summer season, untreated plants and $100_{AN}$ treated plants showed the highest values in terms of TA (Table 3). Treating endive plants with $100_{CC}$ and with $50_{AN}$-$50_{CC}$ decreased TA by 32.0% and 16.0%, respectively, compared to plants supplied with $100_{AN}$. In terms of TA, plants grown in the fall season showed higher values than plants cultivate in the spring-summer season, however, data recorded in the fall season supported the trend established in the spring-summer season (Table 3). Nitrogen source markedly affected ascorbic acid content. Treating endive plants with $100_{CC}$ or with $50_{AN}$-$50_{CC}$ increased ascorbic acid by 17.6% and 8.2%, respectively, compared to plants grown with $100_{AN}$. The lowest ascorbic acid content was recorded in plants grown in absence of nitrogen. In respect to ascorbic acid, data recorded in the fall season supported the trend established in spring-summer season (Table 3).

Plants cultivated in the spring-summer season and supplied with $100_{CC}$, $50_{AN}$-$50_{CC}$ or $100_{AN}$ had a significantly higher TSS than those unfertilized. Whereas, in fall season, plants treated with $100_{AN}$ showed no significant statistical differences compared to the untreated ones (Table 3). Moreover, in both seasons, unfertilized plants gave the lowest TSS value. Plants from the spring-summer season supplied with $100_{CC}$ gave the highest total phenolic content (0.90 mg of caffeic acid $g^{-1}$ fw) followed by those supplied with $50_{AN}$-$50_{CC}$. Unfertilized plants and plant fertilized with $100_{AN}$ gave the lowest total phenolic values, whereas, in fall season, the lowest total phenolic levels were recorded in plants from control plots. Remarkably, in fall season, plants grown on plots treated with $100_{CC}$, $50_{AN}$-$50_{CC}$ or $100_{AN}$ did not show significant differences in terms of total phenolic content.

As regards N content, plants grown in both seasons with $100_{AN}$ gave the highest N content followed by those supplied with $50_{AN}$-$50_{CC}$. In fall season, significantly lower N values were detected in plants supplied with $100_{CC}$. Whereas, in spring-summer season, ANOVA and mean separation did not highlight significant differences between plants treated with $50_{AN}$-$50_{CC}$ and those treated with $100_{CC}$. Finally, data on Nacc supported the trend established for leaves N content (Table 4).

**Table 3.** Effect of nitrogen source on titratable acidity (TA) and ascorbic acid, total soluble solid (TSS) and total phenolic of curly endive grown in spring-summer and fall seasons.

| | Spring-Summer Season | | | | | | | | Fall Season | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Nitroge Source** | **TA (%)** | | **Ascorbic Acid (mg kg$^{-1}$ fw)** | | **TSS (Brix°)** | | **Total Phenolic (mg of Caffeic Acid g$^{-1}$ fw)** | | **TA (%)** | | **Ascorbic Acid (mg kg$^{-1}$ fw)** | | **TSS (Brix°)** | | **Total Phenolic (mg of Caffeic Acid g$^{-1}$ fw)** | |
| 0 | 0.933 | a | 75.70 | d | 3.73 | b | 0.58 | c | 1.17 | a | 73.27 | c | 3.40 | b | 0.49 | b |
| $100_{AN}$ | 0.833 | ab | 87.13 | c | 4.03 | a | 0.63 | c | 1.03 | ab | 79.67 | bc | 3.63 | b | 0.69 | a |
| $50_{AN}$-$50_{CC}$ | 0.700 | bc | 94.27 | b | 4.10 | a | 0.78 | b | 0.90 | b | 83.00 | b | 4.00 | a | 0.69 | a |
| $100_{CC}$ | 0.567 | c | 102.6 | a | 4.20 | a | 0.90 | a | 0.67 | c | 92.20 | a | 4.07 | a | 0.76 | a |
| Significance | *** | | *** | | *** | | *** | | *** | | *** | | *** | | * | |

Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey Honestly Significant Difference (HSD) Test. The significance is designated by asterisks as follows: *** statistically significant differences at *p*–value below 0.001; * statistically significant differences at *p*–value below 0.05; NS, not significant. 0, no nitrogen; $100_{AN}$, fertilized with ammonium nitrate at 100 kg N ha$^{-1}$; $50_{AN}$-$50_{CC}$, fertilized with ammonium nitrate at 50 kg N ha$^{-1}$ and with calcium cyanamide at 50 kg N ha$^{-1}$;$100_{CC}$ fertilized with calcium cyanamide at 100 kg N ha$^{-1}$.

**Table 4.** Effect of nitrogen source on N and $N_{acc}$ of curly endive grown in spring-summer and fall seasons.

| | Spring-Summer Season | | | | Fall Season | | | |
|---|---|---|---|---|---|---|---|---|
| **Nitrogen Source** | **N (mg g$^{-1}$ DW)** | | **$N_{acc}$ (kg ha$^{-1}$)** | | **N (mg g$^{-1}$ DW)** | | **$N_{acc}$ (kg ha$^{-1}$)** | |
| 0 | 1.97 | c | 3.43 | c | 2.34 | d | 5.90 | d |
| $100_{AN}$ | 5.70 | a | 15.73 | a | 6.70 | a | 30.00 | a |
| $50_{AN}$-$50_{CC}$ | 4.13 | b | 12.07 | b | 4.49 | b | 20.30 | b |
| $100_{CC}$ | 3.83 | b | 10.67 | b | 3.36 | c | 15.57 | c |
| Significance | *** | | *** | | *** | | *** | |

Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey Honestly Significant Difference (HSD) Test. The significance is designated by asterisks as follows: *** statistically significant differences at *p*–value below 0.001. 0, no nitrogen; $100_{AN}$, fertilized with ammonium nitrate at 100 kg N ha$^{-1}$; $50_{AN}$-$50_{CC}$, fertilized with ammonium nitrate at 50 kg N ha$^{-1}$ and with calcium cyanamide at 50 kg N ha$^{-1}$; $100_{CC}$ fertilized with calcium cyanamide at 100 kg N ha$^{-1}$.

### *3.3. Nitrogen Efficiency Indices*

In both, spring-summer and fall seasons, nitrogen source did not significantly affect $PFP_N$ and $AE_N$ (Table 5).

**Table 5.** Effect of nitrogen source on $PFP_N$, $AE_N$, PEN and $REC_N$ of curly endive grown in spring-summer and fall seasons.

| | Spring-Summer Season | | | | | | | | Fall Season | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Nitrogen Source** | **$PFP_N$** | | **$AE_N$** | | **$PE_N$** | | **$REC_N$** | | **$PFP_N$** | | **$AE_N$** | | **$PE_N$** | | **$REC_N$** | |
| $100_{AN}$ | 27.6 | a | 10.4 | a | 83.71 | b | 0.124 | a | 44.79 | a | 27.67 | a | 103.14 | c | 0.266 | a |
| $50_{AN}$-$50_{CC}$ | 29.2 | a | 12.1 | a | 138.90 | a | 0.087 | b | 45.23 | a | 28.12 | a | 166.34 | b | 0.170 | b |
| $100_{CC}$ | 27.8 | a | 10.7 | a | 146.84 | a | 0.072 | b | 46.33 | a | 29.22 | a | 239.37 | a | 0.123 | c |
| Significance | NS | | NS | | * | | *** | | NS | | NS | | *** | | *** | |

Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey Honestly Significant Difference (HSD) Test. The significance is designated by asterisks as follows: *** statistically significant differences at *p*–value below 0.001; * statistically significant differences at *p*–value below 0.05; NS, not significant. 0, no nitrogen; $100_{AN}$, fertilized with ammonium nitrate at 100 kg N ha$^{-1}$; $50_{AN}$-$50_{CC}$, fertilized with ammonium nitrate at 50 kg N ha$^{-1}$ and with calcium cyanamide at 50 kg N ha$^{-1}$; $100_{CC}$ fertilized with calcium cyanamide at 100 kg N ha$^{-1}$

The effects of tested treatment on $PE_N$ are presented in Table 5. Plants from the spring-summer season supplied with $100_{CC}$ and with $50_{AN}$-$50_{CC}$ increased $PE_N$ by 75.4% and 65.9%, respectively compared to the plants supplied without $100_{AN}$. Whereas, $PE_N$ in plants from the fall season and supplied with $100_{CC}$ or $50_{AN}$-$50_{CC}$ increased by 132.1% and 61.3%, respectively compared to the plants supplied with $100_{AN}$. Plants both from fall and spring-summer season and fertilized with $100_{AN}$ had the lowest $PE_N$ values.

The effects of the nitrogen source on $REC_N$ are presented in Table 5. In spring-summer season, $REC_N$ was the highest in plants cultivated with $100_{AN}$ and the lowest in plants cultivated with $50_{AN}$-$50_{CC}$ or $100_{CC}$. In fall season, the highest $REC_N$ values were observed in $100_{AN}$ treatment, whereas, the lowest values were recorded from plants from $100_{CC}$ treatment. Data collected in fall season supported the trend established in spring-summer season. Overall, growing endive plants during the spring-summer season substantially reduced $REC_N$ as compared to growing plant in the autumn season. Furthermore, increasing the amount of N provided by $CaCN_2$ from 0 to 100% resulted in a $REC_N$ decrease, although, in respect to the spring-summer season, ANOVA analysis did not show significant differences between plants treated with $50_{AN}$-$50_{CC}$ and those treated with $100_{CC}$.

## 4. Discussion

In this work, we evaluated the potential use of $CaCN_2$ as source of nitrogen to enhance production and quality of curly endive grown in two different seasons. Our outcomes are consistent with those obtained by Adamczewska-Sowińska and Uklańska [25] who, by investigating the effects of different type and doses of nitrogen fertilization on endive, found that marketable yield increased with increasing doses of nitrogen as compared to unfertilized control. However, in our study the types of nitrogen source tested ($CaCN_2$ and standard fertigation) were equally effective in increasing head fresh weight and other parameters such as head height, stem diameter, visual quality, number of leaves and head dry matter when compared to unfertilized control. These results agree with those observed in lettuce, another member of the *Asteraceae* family, by Montemurro et al. [26] who, by comparing dicyandiamide, a nitrification inhibitor (NI), with urea did not find significant differences in terms of yield. Our results are also consistent with those of Di Gioia et al. [13] who found the same leaf area index, fresh yield and dry weight when comparing two lettuce types either fertilized with calcium cyanimide or with a standard split application of soluble nitrogen.

There are some reports demonstrating that applying $CaCN_2$ or other stabilized fertilizers that contain NIs is able to raise the yield in potato [27], soybean [28], maize [29], and wheat [30]. However, as hypothesized by Frye [31], Di Gioia et al. [13] and Sabatino et al. [11] the application of NIs may cause an increase in yield traits only at low N application rates or when N is lost by leaching and/or denitrification, causing a shortage in N level quite strong to reduce crop yield in absence of NI.

Our findings demonstrated that autumn season positively affected yield traits and plant visual quality. Currently, there is a mounting proof indicating the additive and synergetic effects of natural bioactive compounds on human health, especially from plant sources since they are able to diminish the risk of several dysfunctions linked to oxidative stress [32–34]. Our findings demonstrated that, compared with standard fertigation, a $CaCN_2$ pre-transplant treatment, in spring-summer season, can improve the ascorbic acid and polyphenol contents in curly endive. Whereas, the $CaCN_2$ pre-transplant treatment in fall season, positively effects ascorbic acid and TSS content. Our findings are coherent with those acquired by Mercelle [35], Stefanelli et al. [36] and Sabatino et al. [11], who indicated negative effects of high N administrations on nutritional and functional fruit traits. Consequently, we may hypothesize that curly endive plants in plots treated with the highest dose of $CaCN_2$ had higher TSS, ascorbic acid and total phenolic contents, caused by a slow nitrification process defined by $CaCN_2$ application and the remains of ammonium-N in the soil which can take part in reducing nutritional stresses procured by the risks of excesses or N deficiency in specific phases of the growing cycle. Moreover, our data revealed that, except for $100_{AN}$ treatment, spring-summer season positively influenced total phenolic content in curly endive plants. Thus, since stress conditions prompt phenolics accumulation [37–41] and considering that the optimum growth temperature for curly endive is about 15–18 °C [42], we may hypothesize that the higher total phenolic content observed in plants from the spring-summer cycle could be due to the high temperatures (thermal stress), which are usual in the Mediterranean climate regions during spring-summer time.

Our findings showed that, in the fall season, $N_{acc}$ decreased as the percentage of nitrogen supplied by $CaCN_2$ increased. This is in line with the results of Pleysier et al. [43] who, by inquiring the consequences of the application of $CaCN_2$ four weeks before maize and rice plantation, found that $N_{acc}$ decreased with increasing doses of nitrogen supplied via $CaCN_2$. This response was attributed by these authors to N loss by volatilization between application and planting time. Besides, our results demonstrated that autumn growing season positively affected $N_{acc}$ compared to the spring-summer season. This could be related to the fact that autumn temperatures in the Mediterranean region, differently from the tropics, do not reach quite high levels to provoke considerable losses of ammonium-N by volatilization. Furthermore, our results are, also, in accord with those of Colonna et al. [44], who by investigating on the nutritional traits of ten leafy green vegetables harvested at two light intensities, found lower protein and $NO_3$-N content when plants are grown at lower PAR level. Our results concerning NUE indices, either in terms of $REC_N$ or in terms of NUE expressed as

$PE_N$, demonstrated that both growing seasons and nitrogen supplied via $CaCN_2$ play a major role. As regards the NUE expressed as $PFP_N$ and $AE_N$, the application of $CaCN_2$ did not result in any improvement. However, $PFP_N$ and $AE_N$ indices were considerably higher in autumn season than in the spring-summer season. Our findings are in line with those of Chen et al. [12] and with those of Pleysier et al. [43], who reported that $CaCN_2$ or other NI can potentially enhance NUE. Nevertheless, our results are partially coherent with those of Di Gioia et al. [13], who found that applying $CaCN_2$ improved NUE neither in terms of $REC_N$ nor in terms of NUE expressed as $PFP_N$, $AE_N$, and $PE_N$. Since, the experimental fertilization schemes proposed in this study improved imperative features in curly endive, they seem to be feasible for curly endive commercial production in both spring-summer and fall seasons.

## 5. Conclusions

Our study suggests that a pre-transplant application of $CaCN_2$ can be an alternative and valuable source of N for curly endive production. Overall, $CaCN_2$ did not improve crop yield. Nevertheless, $CaCN_2$, especially when used at 100 kg N $ha^{-1}$ ($100_{CC}$), effectively increased ascorbic acid and total phenolic in spring-summer season and ascorbic acid and TSS in fall season. Simultaneously, $CaCN_2$ pre-transplant treatment reduced N content and $N_{acc}$. Furthermore, $CaCN_2$ improved NUE indices both in terms of $REC_N$ and $PE_N$. Our results also showed that autumn season increased yield traits, plant visual quality, ascorbic acid content and NUE indices as compared to the spring-summer season.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2073-4395/10/9/1357/s1, Table S1: Significance of two-way ANOVA analysis (nitrogen source × year).

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