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Nitrogen Source and Dose Effects on Spinach Yield and Total Nitrogen Content Across Storage Periods

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Abstract
Spinach (*Spinacia oleracea* L.) cultivation is gaining prominence due to increasing interest in health and wellness. Nitrogen (N) is crucial for spinach growth, and traditional fertilization practices are being reconsidered for their environmental impact. In this study, we investigated the effects of different N sources, doses (25, 50, 100, 200, and 400 mg N kg^{-1}), and storage durations on dry weight and total N content of spinach plants. Four N sources, including ammonium sulfate (AS), calcium nitrate (CN), slow-release ammonium soil (SRAS). The spinach plants were subjected to storage for 0, 5, and 10 days, and their dry weight and total N content were measured. The results revealed significant variations in spinach dry weight and total N content among different nitrogen treatments and storage periods. Calcium nitrate consistently promoted higher dry weight and total N content, reaching 5.40 % for total N at 400 mg kg^{-1} on Day 0, compared to other N sources across various storage durations. The YR and SRAS also showed high total N levels, with values consistently above 4 % at the highest dose. The SRAS exhibits potential for prolonged efficacy, particularly at higher doses and later storage stages. Initially, at Day 0, the total N content in SRAS treatment was highest and decreased significantly by Day 5, with a slight recovery by Day 10. The correlation between dry weight and total N content became more pronounced with longer storage periods and higher N doses and sources. Yeast residue exhibited the strongest positive correlation between dry weight and total N content, suggesting its effectiveness in promoting dry weight. In addition, a strong positive correlation between dry weight and total N content underscores the crucial role of N management in determining spinach yield and quality. The results showed that CN and SRAS, particularly at higher doses, were the most effective N sources for promoting dry weight in spinach. The findings underscore the importance of selecting the appropriate N source and dose is crucial for optimizing spinach yield and quality during storage.

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1. Introduction

Spinach (*Spinacia oleracea* L.) is a nutritious leafy green vegetable from the Chenopodiaceae family, renowned for its rapid growth cycle (Naseem et al., 2024). The spinach contains a wealth of vitamins A, B, and C, alongside essential minerals (Tai et al., 2020). Driven by a growing global population focused on health and wellness, spinach has emerged as a vegetable of increasing interest. This surge in popularity is reflected in the increased spinach production in the world. Cultivated extensively around the globe, spinach boasts an annual production of 30 million tons. China reigns supreme in global production, contributing a staggering 27.5 million tons. Following China, the United States (435.721 tons) and Japan (226.865 tons) secure the second and third positions, respectively. Türkiye holds the fourth place with an annual spinach production of 229.793 tons (Anonymous, 2024).

Nitrogen (N) is the most essential element for healty spinach growth and productivity. This assertion is supported by a robust body of research demonstrating a significant influence of N on these key aspects. During the vegetative stage, increasing the application of N levels can empower plants in two ways. First, N improves and supports root development, allowing for increased water and nutrient absorption. Second, it accelerates growth, resulting in an abundance of lush, green foliage. This translates to potentially higher yields, improved vegetable flavor, and even enhanced resistance to pests, diseases, and environmental stresses (Echert, 2020). In addition, N plays a vital role in plant metabolism, influencing enzyme activity, protoplasm formation, and the synthesis of amino acids and proteins. These processes drive cell division and initiate meristematic activity, impacting overall growth and production (Mirdad, 2009; Kuş and Gedik, 2023).

Mineral nutrients containing forms of N that are easily absorbed by plants are widely used in agriculture. Spinach cultivation practices typically involve the application of inorganic N fertilizers at a rate of approximately 150 kg N ha^{-1}, which indicates the critical role of N in spinach growth and quality (Darani et al., 2013; Rouphael et al., 2018). The long-term effects of slow-release fertilizers, which have gained popularity in recent years and aim to mitigate the negative impacts of chemical fertilizers, have been extensively investigated in various plant species. These fertilizers possess extended efficacy due to their gradual dissolution and prevent nutrient leaching losses. They remain unaltered in the soil, do not bind to clay, lime, or organic matter, and do not form complexes with other elements. Consequently, they are readily absorbed by plants without undergoing conversion to inactive forms (Mukherjee et al., 2015). This enhances fertilizer uptake efficiency. However, in slow-release fertilizers, the nutrient element release over time cannot be accurately predicted, and the release rate varies depending on environmental, soil, and climatic conditions. Nutrient elements in slow-release fertilizers are gradually released, altering the composition of the soil solution as they become available (Azeem et al., 2014). Despite the existence of a diverse array of nitrification inhibitor compounds globally, dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) stand out as the most widely commercially available nitrification inhibitors (Şahan et al., 2017). The primary objective of employing nitrification inhibitors is to prolong the retention of N in the soil in the NH_4^+ form, thereby preventing N losses in the form of NO_3^- or gaseous N and enhancing N utilization efficiency (Trenkel, 2010). Slow-release fertilizers offer several advantages, including the ability to be applied in a single application, minimize nutrient element losses, reduce the detrimental impacts on human health associated with soil respiration, and enhance fertilizer use efficiency (Hutchinson et al., 2002: Sempeho et al., 2014). In their study on N transformations in soil and plant productivity, Yang et al. (2016) conducted a meta-analysis on the effectiveness of two different nitrification inhibitors [dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP)]. They concluded that both DCD and DMPP were equally effective in regulating soil N transformations in agricultural fields worldwide. However, they highlighted that DCD exhibited a superior ability to enhance plant productivity compared to DMPP. Additionally, they emphasized the need for further research to evaluate the long-term performance of DCD and DMPP under continuous application.

Organic amendments are emerging as compelling alternatives to slow-release and chemical fertilizers in sustainable agricultural practices. Despite their lower nutrient content, organic fertilizers play a pivotal role in enhancing soil organic matter and improving soil physical properties. Their multifaceted positive contributions to the soil include accelerating microbiological activity, enhancing soil structure, aeration, and water holding capacity, and providing both macro- and micronutrients (Kara and Yakupoğlu, 2023). A study by Cıtak and Sonmez (2010) compared the effects of three organic manure applications and their mixtures against conventional chemical fertilizers and an unfertilized control plot on spinach growth, yield, and nutrient content across two seasons. Their findings highlight the potential effectiveness of organic amendments, particularly in the autumn season, where all organic applications demonstrated positive impacts on spinach growth compared to the winter season.

Waste organic matter produced after yeast production can be used as feed in animal husbandry and as the organic main raw material in the production of organic liquid fertilizer in agriculture. A comprehensive review of existing literature revealed a dearth of studies that directly comparethe effects of chemical, slow-release, and yeast production waste fertilizers on spinach growth and total N content. Therefore, this study was conducted to investigate the changes in dry matter yield and total N content during a 10-day storage period in spinach grown under different fertilizer sources and increasing dosage applications.

2. Materials and Methods

2.1. Material

This study was conducted in the Research and Application Greenhouses of the Department of Soil Science and Plant Nutrition at Çukurova University Faculty of Agriculture. The experiment was established in February, and Matador spinach (*Spinacia oleracea* L.), a broad-leaved variety, was used as the plant material. The variety is characterized by rapid growth, full leaves, short stems, a dark green color, a smooth surface, and oval leaf tips that spread out over the soil. It is cultivated in all regions of Türkiye and is both productive and cold tolerant. The recommended spacing between rows is 5-10 cm, the spacing within rows is 30-35 cm, and the planting depth is 1.5-2.5 cm. The optimum temperature for germination and growth is 15-25°C.

2.2. Methods

2.2.1. Greenhouse experiment setup and implementation

The greenhouse experiment was conducted using plastic pots with 2 kg of soil per pot. As base fertilization, KH_2PO_4 was applied to provide 100 mg P kg^{-1}, 125 mg K kg^{-1}, and Fe-EDTA was applied to provide 10 mg Fe kg^{-1}, while $ZnSO_4$ was applied to provide 2.5 mg Zn kg^{-1}. The amount of $CaSO_4$ was calculated to ensure equal sulfur content in all pots based on the sulfur content of the ammonium sulfate applied. Ten seeds were sown in each pot and thinned to six seedlings after germination.

The experiment consisted of 60 pots with five different N doses (25, 50, 100, 200, and 400 mg N kg^{-1}). The increasing doses of N were applied from slow-release ammonium sulfate (SRAS) (DCD Inhibitor), ammonium sulfate $(NH_4)_2SO_4$ (AS), calcium nitrate $Ca(NO_3)_2$ (CN), and yeast residue (YR). The experiment was designed in a randomized block design with three replications.

Seeds were sown in the pots on February 24, 2021. Initial germination occurred within 8-10 days, and approximately 16 days later (March 10, 2021), the 10 seedlings in each pot were thinned to six seedlings. The pots were watered to maintain field capacity from seed sowing until harvest. Growth and development were monitored throughout the experiment. Plants were harvested at 52 days of the experiment on April 16, 2021, when significant differences in growth and development were observed due to the different N fertilizer sources and increasing N application rates.

Following the harvest at 52 days of the experiment, the harvested plants were divided into three equal portions for further analysis. The first portion served as the initial sample (Day 0 period) and was washed and dried immediately in a 48 °C oven. The second portion (Day 5 period) was washed and stored in a sealed polyethylene bag for five days at refrigeration temperature (+4 °C). Similarly, the third portion (Day 10 period) was washed and stored in a refrigerator for ten days. After their respective storage periods, all plant samples were dried thoroughly in a 70 °C oven for 48 hours for further analysis.

2.2.2. Physical and chemical analysis of expeirmental soil

The soil used in the experiment was sieved (2 mm) and analyzed for various physical and chemical properties using established methods. Electrical conductivity and pH were measured with a pH meter in a 1:2.5 soil-water dilution (Mclean et al., 1982). Lime content ($CaCO_3$) was determined by the Scheibler calcimeter method (Allison and Moodie, 1965). Available Zn, Fe, Mn, and Cu were extracted with DTPA-TEA solution (pH 7.3) suitable for calcareous soils (Lindsay and Norvell, 1978). Organic matter content was assessed using the Walkey-Black wet digestion method (Jackson, 1959). Available phosphorus was determined based on molybdenum blue colorimetry after extraction with 0.5 M $NaHCO_3$ solution (Olsen et al., 1954). Finally, available potassium and magnesium were extracted with 1 N ammonium acetate solution following the method developed by Knudsen et al. (1982).

2.2.3. Total N analysis in plants

Total N content of spinach samples was determined using the Kjeldahl distillation method (Bremner, 1965). The principle of this method is to convert organic N in the fresh-burned plant sample with H_2SO_4 into NH_4-N form, and to determine the N content of the plants from the amount of NH_3 released and captured in boric acid during distillation conducted in an alkaline environment.

2.2.4. Statistical analysis

Statistical analysis of the data was conducted using the SPSS software. To assess the effects of different N sources and doses on dry weight and total N content of spinach plants during 10 days of storage period, analysis of variance (ANOVA) was performed. The least significant difference (LSD) test was applied to determine the significance of differences between means. All statistical analyses were set at a significance level of $P<0.05$. In addition, correlations between dry weight and total N content of spinach plants were carried out to assess nitrogen use efficiency. This analysis helps us understand how effectively the spinach plants are utilizing nitrogen for growth.

3. Results and Discussion

3.1. The effect of different N sources, doses and duration of starogae on dry matter yield

Table 1 presents the effects of various N sources and application rates on spinach dry weight during different storage periods (Day 0, 5, and 10). The ANOVA results showed statistically significant differences in dry weight based on day, fertilizer type, and N dose (p-values of 0.004, 0.003, and 0.001, respectively). However, the interactions between day, fertilizer type, and N dose were not statistically significant, suggesting the effects of N source and dose were consistent across the storage periods.

The highest dry weight in Day 0 was obtained with the 200 mg N kg^{-1} dose of SRAS (2.17±0.32 g $plant^{-1}$) and the 400 mg N kg^{-1} dose of YR (2.07±0.14 g $plant^{-1}$), both showing significant increases in dry weight. The lowest dry weight was observed with the 25 mg N kg^{-1} dose of YR (1.04±0.16 g $plant^{-1}$). The 400 mg N kg^{-1} dose of CN (2.26±0.51 g $plant^{-1}$) resulted in the highest dry weight in Day 5, followed by SRAS (2.13±0.11 g $plant^{-1}$). The lowest dry weight was recorded with the 25 mg N kg^{-1} dose of YR (0.85±0.05 g plant^{-1}). The highest dry weight in Day 10 was seen with the 400 mg N kg^{-1} doses of SRAS (2.78±0.10 g plant^{-1}), CN (2.59±0.51 g plant^{-1}), and YR (2.76±0.26 g plant^{-1}). The lowest dry weight was observed with the 25 mg N kg^{-1} dose of AS (1.14±0.41 g plant^{-1}).

Table 1. Effects of different N sources and doses on dry weight of spinach during different storage periods

Period	N dose	Ammonium Sulphate	SRAS	Calcium Nitrate	Yeast Residue
	mg/kg	g plant^{-1}			
Day 0	25	1.24±0.04 h-k	1.41±0.61 e-k	1.34±0.22 g-k	1.04±0.16 jk
	50	1.42±0.24 e-k	1.48±0.16 e-k	1.44±0.10 e-k	1.32±0.02 g-k
	100	1.46±0.14 e-k	1.68±0.14 d-k	2.03±0.20 a-i	1.78±0.33 b-j
	200	1.98±0.14 a-i	2.17±0.32 a-g	1.83±0.56 b-j	1.80±0.11 b-j
	400	1.62±0.22 d-k	2.06±0.03 a-h	1.67±0.12 d-k	2.07±0.14 a-h
Day 5	25	1.44±0.21 e-k	1.14±0.13 ijk	1.36±0.07 f-k	**0.85±0.05 k**
	50	1.52±0.38 d-k	1.49±0.10 e-k	1.50±0.20 d-k	1.25±0.19 h-k
	100	1.83±0.40 b-j	2.28±0.45 a-e	2.02±0.29 a-i	1.49±0.22 e-k
	200	2.03±0.16 a-i	1.90±0.21 a-j	1.51±0.22 d-k	1.46±0.10 e-k
	400	1.99±0.13 a-i	2.13±0.11 a-h	2.26±0.51 a-f	1.79±0.02 b-j
Day 10	25	1.14±0.41 ijk	1.28±0.09 g-k	1.75±0.05 b-j	1.24±0.13 h-k
	50	1.59±0.20 d-k	1.78±0.13 b-j	2.0±0.51 a-i	1.33±0.14 g-k
	100	1.75±0.35 b-j	1.65±0.15 d-k	2.09±0.23 a-h	1.78±0.14 b-j
	200	1.72±0.42 c-k	2.39±0.20 a-d	2.61±0.15 ab	1.63±0.12 d-k
	400	1.92±0.19 a-j	**2.78±0.10 a**	2.59±0.51 abc	**2.76±0.26 a**
ANOVA	Day		LSD = 0.154 P = 0.004		
	Fertilizer Type		LSD = 0.183 P = 0.003		
	N Dose		LSD = 0.204 P = 0.001		
	Day x Fertilizer Type		LSD = 0.316 P = 0.074		
	Day x N Dose		LSD = 0.354 P = 0.219		
	Fertilizer Type x N Dose		LSD = 0.409 P = 0.627		
	Day x Fertilizer Type x N Dose		LSD = 0.708 P = 0.934		

* Values with the same letter in the table do not significantly differ from each other ($p<0.05$).

The highest dry weight in AS treatments was observed at 200 mg kg^{-1} for all storage days (Day 1: 1.98 g plant^{-1}, Day 5: 2.03 g plant^{-1}, Day 10: 1.72 g plant^{-1}). The increase in N dose (400 mg kg^{-1}) resulted in a decrease or stagnation in dry weight compared to 200 mg kg^{-1} across all storage days. Unlike AS, SRAS displayed a slightly different pattern. While the highest dry weight on Day 0 was at 200 mg kg^{-1} (2.17 g plant^{-1}), the highest on Day 10 was at 400 mg kg^{-1} (2.78 g plant^{-1}). The SRAS at 400 mg kg^{-1} maintained a statistically similar or slightly higher dry weight compared to 200 mg kg^{-1} on some storage days (Day 5 and 10), suggesting a potential benefit for this dose at later storage stages.

The results show a dose-dependent increase in the dry weight of spinach plants with increasing doses of calcium nitrate over different storage periods. This trend is consistent with the results reported by Elsayed and Abdelraouf (2016), who found that increasing N fertilization rates up to 224 kg N ha^{-1} in spinach increased plant growth, fresh and dry weight, leaf area, and leaf area index. Calcium nitrate at higher doses (200 mg kg^{-1} and 400 mg kg^{-1}) consistently led to greater dry weight compared to lower doses. This suggests that CN is an effective N source for promoting growth in spinach plants. The highest dry weight on Day 0 was observed with the application of 100 mg kg^{-1} (2.03±0.20 g plant^{-1}). Like Day 0, the dry weight of spinach plants increased with the increase in CN doses on Day 5. At 25 mg kg^{-1}, the dry weight was 1.36±0.07 g plant^{-1}, whereas at 400 mg kg^{-1}, it reached the highest value at 2.26±0.51 g plant^{-1}. On Day

10, the dry weight continued to follow a dose-dependent trend. The dry weight on Day 10 was 1.75±0.05 g plant^{-1} at 25 mg kg^{-1} and increased to the highest level of 2.61±0.15 g plant^{-1} at 200 mg kg^{-1}. The 400 mg kg^{-1} dose resulted in a slightly lower value of 2.59±0.51 g plant^{-1}. Karaal and Uğur (2014) observed a similar trend, with their average dry matter yield increase ranging from 17.4 % to 66.1 % for increasing N doses compared to the N25 application. This further supports the notion that N dosing plays a crucial role in maximizing spinach yield and quality.

In optimizing spinach yield and quality, selecting the right N source is essential (Zhang et al., 2018). Our research investigated the impact of various N sources on dry weight, and the findings align with observations from previous studies. Krezel and Kolota (2014) explored the influence of different N sources on spinach, and their results agreed with our findings. They recorded the highest yield with ENTEC 26 - DMPP, while AS resulted in the lowest. This highlights the significant variation in yield depending on the N source used. In our study, a moderate dose (200 mg kg^{-1}) of AS and SRAS exhibited a positive impact on dry weight up to a certain point. However, AS displayed a decrease or stagnation in dry weight with increasing storage days, suggesting a potential limitation in shelf life. SRAS, on the other hand, showed promise for later storage, particularly at the higher dose (400 mg kg^{-1}) on Day 10, where it maintained or even increased dry weight compared to the 200 mg kg^{-1} dose.

The research conducted to investigate the impact of the DMPP inhibitor on nitrate (NO_3^-) concentration and yield in spinach grown in various soil types revealed that the SRAS application significantly increased leaf yield by 29 % compared to the standard AS application (Ghetasi and Hosseinpur, 2020). Moreover, other studies, such as one by Mordoğan et al. (2001), have shown positive effects of increasing N doses on lettuce yield. Chohura and Kolota (2011) further indicated that yield in lettuce varies depending on the specific N sources and varieties used. Considering previous findings, we observed serious problems in plant dry matter yield particularly when the 25 mg N kg^{-1} was used. Conversely, when N was applied at an optimal level, the impact of different N fertilizer sources on increasing dry matter yield became apparent. It is important to note that both the application of various N sources and the use of increasing N doses had statistically significant effects ($P<0.01$) on dry weight (Table 1).

When storage days were not considered, the highest dry weight of spinach plants (2.32 g plant^{-1}) was obtained with SRAS treatment as a dose of x 400 mg N kg^{-1}. This was followed closely by yeast residue (2.20 g plant^{-1}) and calcium nitrate (2.18 g plant^{-1}) both applied at the same dose. These results suggest that high doses of these fertilizers, particularly SRAS, can lead to significant increases in spinach dry weight. In contrast to our findings, Machado et al. (2020) who investigated the impact of N source on spinach shoot dry weight, reported that the N fertilizer source itself did not significantly affect shoot dry weight. The researchers did observe that plants grown solely with organic compost had lower shoot dry weight compared to those receiving compost and additional N fertilization. However, the addition of N (regardless of source) led to a decrease in shoot dry weight percentage. Several factors might contribute to the discrepancies between our findings and those of Machado et al. (2020) such as specific N sources, overall nutrient balance, or cultivar variation. The types of N fertilizers used in our study (SRAS, yeast residue, calcium nitrate) might differ in their uptake and utilization by spinach plants compared to ammonium nitrate used by Machado et al. (2020). The presence or absence of other essential nutrients in the growth medium (beyond N) could influence the response to N application (Aulakh et al., 2005). In our study, the specific fertilizer formulations might have provided a more balanced nutrient profile compared to the sole addition of N in Machado et al. (2020). Different spinach cultivars might exhibit varying responses to N fertilization (Ahmadi et al., 2010; Joshi et al., 2022).

The Figure 1 illustrates the dry weight of spinach plants treated with various N sources, highlighting noticeable variations among the fertilizer types. Calcium Nitrate emerges as the most effective, yielding the highest dry weight at 1.868 g $plant^{-1}$, followed closely by SRAS with 1.841 g $plant^{-1}$. In contrast, AS displays a slightly lower dry weight of 1.642 g $plant^{-1}$, while YR exhibits the lowest dry weight at 1.572 g $plant^{-1}$. The SRAS resulted in a slightly higher dry weight (12.12 % increase) compared to plants grown with AS. The slow-release nature of SRAS could explain this observation. By gradually releasing N over time, SRAS might provide a more continuous and balanced supply of nutrients for plant growth compared to the potentially quicker release of N from AS. This could lead to more efficient N utilization by the spinach plants and consequently, higher dry weight accumulation (Anonymous, 2024). These findings, without considering the N doses and storage days, indicate that CN significantly outperforms the other N sources in promoting spinach dry weight, suggesting its potential as a superior fertilizer option for spinach cultivation.

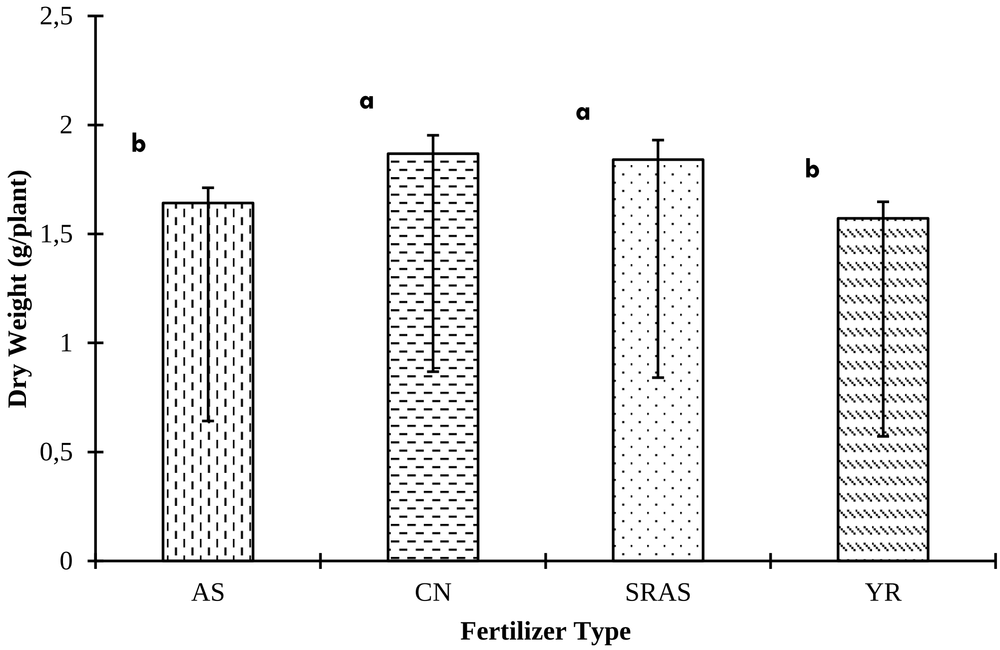

Figure 1. The effects of fertilizer type on dry weights of spinach plants regardless of N doses and duration of storage day. AS: Ammonium Sulphate, CN: Calcium Nitrate, SRAS: Slow-Release Ammonium Nitrate, YR: Yeast Residue

When fertilizer type and storage days were not considered, the results demonstrated a clear trend of increasing dry weight with increasing N dose (Figure 2). At the lowest dose of 25 mg kg^{-1}, the dry weight is 1.269 g, while at the highest dose of 400 mg kg^{-1}, the dry weight reaches 2.136 g, nearly doubling the initial weight. This significant increase in dry weight as N dose increases suggests a positive correlation between N availability and plant growth. The increase in dry matter content was particularly pronounced in CN (Figure 3) and SRAS (Figure 4) applications, with a significant rise in dry matter observed with increasing doses.

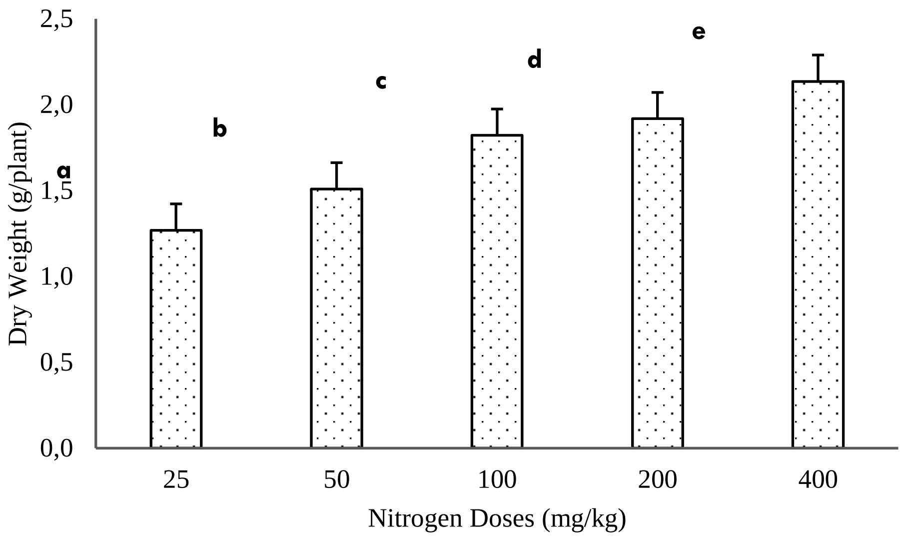

Figure 2. The effects of N doses on dry weights of spinach plants regardless of N sources and duration of storage day

Figure 3. The increased doses of $Ca(NO_3)_2$ on growth of spinach plants

Figure 4. The increased doses of slow erleased ammonium sulphate fertilizer on growth of spinach plants

3.2. The effect of different n sources, doses and duration of starogae on total N content

The effects of different N sources (Ammonium Sulfate, SRAS, calcium nitrate, yeast residue) and doses (25 mg kg^{-1} to 400 mg kg^{-1}) on the total N content of spinach plants during storage periods (Day 0, 5, and 10) are presented in Table 3. The table reports a statistically significant impact of storage day, fertilizer type, and N dose (p-values < 0.001 for each factor) on the total N content of spinach plants. Additionally, significant interactions were observed between these factors (p-values < 0.001 for Day x Fertilizer Type, Day x N Dose, Fertilizer Type x N Dose, and Day x Fertilizer Type x N Dose).

Table 2. Effects of different N sources and doses on total N content of spinach during different storage periods

Period	N dose	Ammonium Sulphate	SRAS	Calcium Nitrate	Yeast Residue
	mg kg^{-1}	%			
Day 0	25	**1.15 aa-b ±0.08**	1.73 w-z±0.08	1.91 s-w±0.09	2.15 n-s±0.01
	50	1.26 aa ±0.04	1.95 r-w±0.02	1.98 q-v±0.05	2.17 m-r±0.02
	100	2.18 l-r ±0.03	2.16 n-s±0.09	2.02 p-v±0.03	2.18 l-r±0.01
	200	2.39 i-n ±0.11	3.06 f±0.09	3.70 d±0.17	3.31 e±0.02
	400	2.63 gh ±0.04	4.75 c±0.15	**5.40 a±0.08**	4.57 c±0.05
Day 5	25	1.82 v-y±0.06	1.63 yz±0.09	**1.12 aa-b±0.03**	1.01 ab±0.01
	50	2.08 o-u±0.06	1.65 xyz±0.10	**1.16 aa-b±0.03**	1.28 aa±0.04
	100	2.20 l-r±0.02	1.86 u-y±0.08	1.66 xyz±0.02	1.88 t-x±0.07
	200	3.76 d±0.17	2.27 k-p±0.17	2.13 o-s±0.08	1.96 r-w±0.07
	400	4.98 b±0.04	2.59 ghi±0.12	3.693 d±0.10	2.41 h-m±0.15
Day 10	25	1.53 z±0.01	2.07 o-u±0.02	2.19 l-r±0.04	2.48 h-k±0.02
	50	2.12 o-t±0.03	2.49 h-k±0.06	2.20 l-r±0.01	2.52 g-j±0.03
	100	2.19 l-r±0.02	2.64 gh±0.07	2.21 l-q±0.02	2.14 o-s±0.03
	200	2.32 j-o±0.03	2.29 j-o±0.03	2.49 h-k±0.03	2.47 h-k±0.05
	400	2.13 o-s±0.01	2.75 g±0.02	2.42 h-l±0.17	2.49 h-k±0.05
ANOVA	Day		LSD = 0.046 P = 0.001		
	Fertilizer Type		LSD = 0.053 P = 0.001		
	N Dose		LSD = 0.059 P = 0.001		
	Day x Fertilizer Type		LSD = 0.092 P = 0.001		
	Day x N Dose		LSD = 0.103 P = 0.001		
	Fertilizer Type x N Dose		LSD = 0.119 P = 0.001		
	Day x Fertilizer Type x N Dose		LSD = 0.206 P = 0.001		

* Values with the same letter in the table do not significantly differ from each other ($p<0.05$).

As expected, increasing N dose (from 25 mg kg^{-1} to 400 mg kg^{-1}) led to higher total N content on Day 0. However, the relationship between dose and total nitrogen N might be more complex at later storage days (Day 5 and 10) based on the observed interactions (Table 3). The data implies that different fertilizer types may affect N uptake in spinach plants differently. This could be due to variations in the chemical composition of each fertilizer type, its release rate, or its compatibility with the soil and plant requirements.

When focusing solely on the N dose, excluding the source of N and storage duration, the expected increase in total N content in spinach plants becomes evident (Figure 5). This increase aligns with established findings that higher N doses typically result in greater nutrient absorption in plants. Similar to Zikalala et al. (2017), who reported a significant increase in the percentage N content of baby spinach leaves with increasing N application, our study demonstrated a statistically significant rise in total N content with higher N doses ($p<0.001$) (Table 2). This strengthens the evidence for a direct and robust relationship between N application and plant nutrient uptake. This correlation highlights the importance of managing N dosage carefully to optimize plant health and productivity while minimizing potential negative environmental impacts. Compared to the lowest dose of 25 mg kg^{-1} (1.73 % total N), the 50 mg kg^{-1} dose shows a 10.0 % increase in total N content (1.90 %), the 100 mg kg^{-1} dose shows a 21.9 % increase (2.11 %), the 200 mg kg^{-1} dose shows a 54.8 % increase (2.68 %), and the 400 mg kg^{-1} dose shows a 96.4 % increase (3.40 %) (Figure 2). Our results align with the findings reported by Elsayed and Abdelraouf (2016) concerning the impact of increasing N fertilization rates on total N content and nutrient uptake in spinach plants. Elsayed and Abdelraouf (2016) found that increasing N fertilization rates significantly ($p<0.01$) increased the total N content of spinach, with the highest content observed at a rate of 224 kg N ha^{-1}. Additionally, their study demonstrated a significant increase in N uptake with higher N rates, with the greatest relative increases in N uptake at 112, 168, and 224 kg N ha^{-1} compared to the lowest rate of 56 kg N ha^{-1}.

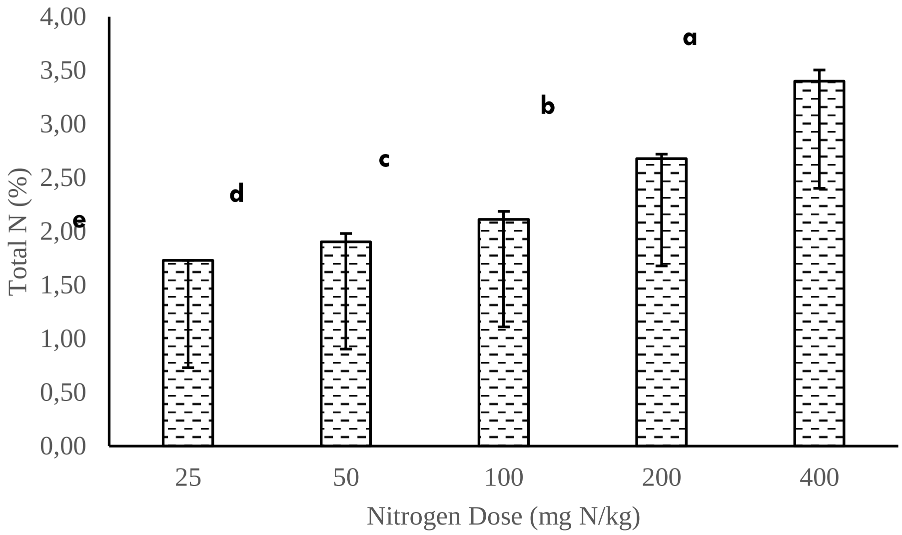

Figure 5. The effects of N dose on total N content of spinach plants regardless of N sources and duration of storage day

The highest overall total N content of spinach plants was recorded with calcium nitrate at 400 mg kg^{-1} on Day 0 (5.40 %). The increase in total N content from 25 mg N kg^{-1} (1.15 %) to 400 mg N kg^{-1} (5.40 %) was 182.7 %. Yeast residue also exhibited high total N levels, reaching 4.57 % at 400 mg kg^{-1} on Day 0 (112.6 % increase) and remaining consistently high across storage days. The highest total N content in SRAS treatments (4.75 %) was observed at the 400 mg kg^{-1} dose on Day 0 (129.6 % increase). Ammonium sulphate followed a similar trend, with the highest content (4.98 %) occurring at 400 mg kg^{-1} on Day 5. The highest values indicate a substantial absorption of N from the soil or fertilizer sources. This high value suggests that conditions were favorable for N uptake and assimilation by the plants. The lowest total N content across all fertilizers and storage days was observed with ammonium sulfate at 25 mg kg^{-1} on Day 5 (1.82 %). Other fertilizers also displayed their lowest content at 25 mg kg^{-1} on Day 5, with yeast residue (1.01 %) and calcium nitrate (ranging from 1.12 % to 1.16 %) exhibiting the lowest values. Unlike other fertilisrs, SRAS displayed its lowest content on Day 0 (1.73 %) at the 25 mg kg^{-1} dose. The lowest total N content suggests limited N uptake, which may indicate potential nutrient deficiencies or suboptimal growing conditions.

Regardless on N doses and duration of storage period, all fertilizer types result in relatively similar total N contents in spinach plants, with CN having the highest average total N content (2.419 %) and YR having the lowest (2.334 %), although the differences are not substantial. SRAS and AS have moderate total N contents (2.392 % and 2.316 %, respectively) (Figure 6).

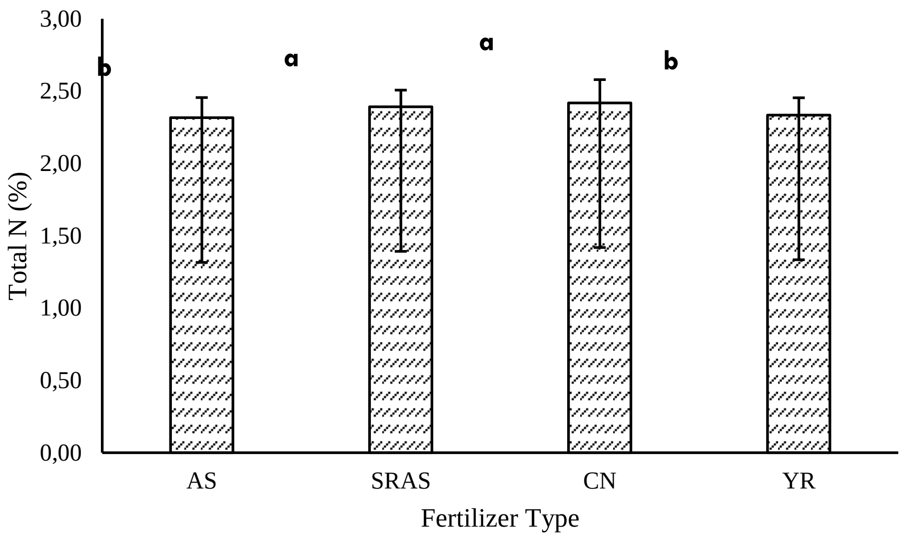

Figure 6. The effects of N dose on total N content of spinach plants regardless of N sources and duration of storage day

Figure 7 shows the total N content in spinach plants over three different storage periods (Day 0, Day 5, and Day 10) regardless of N sources and nitrogen doses. Initially, at Day 0, the total N content was at its highest, measuring 2.633 %. This value decreased significantly by Day 5, reaching a level of 2.155 %, indicating a drop of approximately 18.2 %. By Day 10, the total N content recovered to 2.307 %, representing a slight increase of around 7 % from Day 5 but still lower than the initial level measured at Day 0. The decrease in total N content could potentially lead to a reduction in the overall nutritional value of the spinach during storage. Since N is a crucial component of proteins and other essential plant compounds, this could be a point of concern for maintaining the quality of stored spinach.

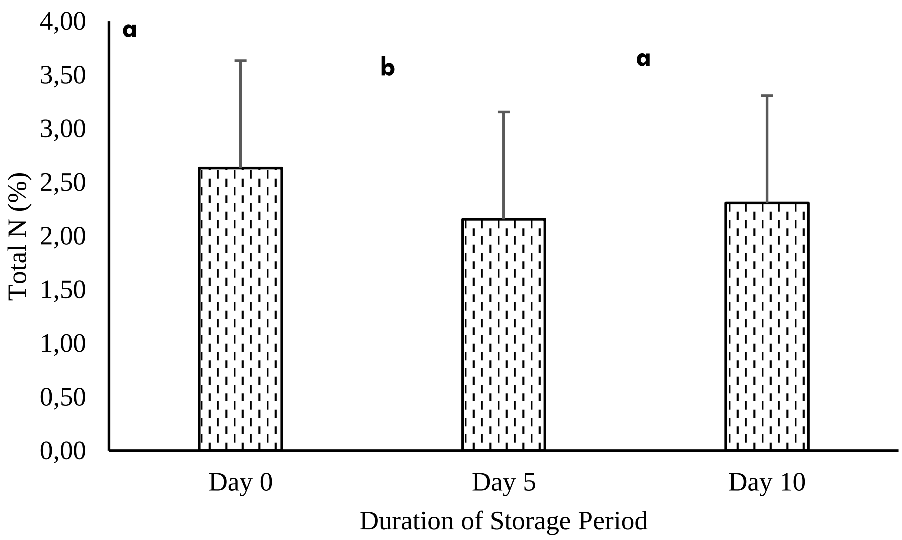

Figure 7. The effects of the duration of storage period on total N content of spinach plants regardless of N sources and N doses

The correlation coefficients between dry weight and total N content of spinach plants according to the N dose used in the spinach growth are given in Table 3. The correlation of 0.457 for 25 mg N kg^{-1} is significant but lower compared to other doses, indicating a moderate positive relationship. At this low dose, N content may not play as prominent a role in influencing dry weight. In 50 mg N kg^{-1} dose, the correlation increased to 0.610, showing a stronger relationship. This suggests that as N dose increases, its impact on dry weight becomes more substantial. In 100 mg N kg^{-1} dose, the correlation further rose to 0.773, indicating a strong positive relationship. This dose is particularly effective at increasing dry weight in correlation with total nitrogen content. In 200 mg N kg^{-1} dose, the correlation drops slightly to 0.607, but remains significant, suggesting that although there is a strong relationship, it is not as pronounced as at the 100 mg N kg^{-1} dose. In 400 mg N kg^{-1} dose, the correlation (0.748) is strong and significant, showing a clear positive relationship. This higher dose may indicate a potential threshold where additional N increases may not yield substantial dry weight gains. Zhang et al. (2014) conducted a field experiment where a moderate N fertilizer application (170 kg ha^{-1}) resulted in the highest spinach yield, while excessive fertilization (255 kg ha^{-1}) had a negative impact. Our findings, coupled with yield response observations from Zhang et al. (2014), emphasize the importance of identifying the optimal N application rate for maximizing spinach dry weight.

Table 3. Correlations between Dry Weight of Spinach Plants and Total N Content

		Dry Weight	Total N
Storage Days			
Day 0	Dry Weight	1	
	Total N	0.740**	1
Day 5	Dry Weight	1	
	Total N	0.912**	1
Day 10	Dry Weight	1	
	Total N	0.924**	1
N Sources			
Ammonium Sulphate	Dry Weight	1	
	Total N	0.793**	1
Calcium Nitrate	Dry Weight	1	
	Total N	0.755**	1
Slow-Release Ammonium Sulphate	Dry Weight	1	
	Total N	0.768**	1
Yeast Residue	Dry Weight	1	
	Total N	0.851**	1
N Doses			
25 mg N/kg	Dry Weight	1	
	Total N	0.457**	1
50 mg N/kg	Dry Weight	1	
	Total N	0.610**	1
100 mg N/kg	Dry Weight	1	
	Total N	0.773**	1
200 mg N/kg	Dry Weight	1	
	Total N	0.607**	1
400 mg N/kg	Dry Weight	1	
	Total N	0.748**	1

** Correlation is significant at the 0.01 level

4. Conclusion and Recommendations

This study investigated the effects of N management on spinach (*Spinacia oleracea* L.) during 10 days of storage period. Nitrogen source, dose, and their interaction significantly impacted spinach quality, including dry weight and total N content. Across various storage periods, calcium nitrate consistently emerged as an effective N source, promoting higher dry weight and total N content compared to other sources. The slow-release nature of some N sources, such as slow-release ammonium sulfate, demonstrated potential benefits for sustained nutrient release and prolonged plant growth, especially for plants with longer storage durations. For instance, SRAS at 400 mg kg^{-1} maintained a statistically similar or slightly higher dry weight compared to 200 mg kg^{-1} on some storage days (Day 5 and 10), suggesting a potential benefit for this dose at later storage stages. Additionally, our findings underscore the importance of N dose optimization, with higher doses leading to increased dry weight and total N content, up to a certain threshold beyond which further increases may not yield significant gains. The correlation analyses further elucidated the relationship between N content and plant growth, emphasizing the critical role of N in determining spinach yield and quality.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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