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Accurate Nitrogen and Water Deficit Trigger Flavonoid and Proline Accumulation in Celery (*Apium graveolens* L.)

Nitrogen dan Kekurangan Air Meningkatkan Akumulasi Flavonoid dan Prolin pada Seledri (Apium graveolens L.)

Andi Kurniawan, Cicik Udayana, Nadya Inri Meiana M., Salvia Salsabila, and Nunun Barunawati*
Department of Agronomy, Faculty of Agriculture, Universitas Brawijaya
Jl. Veteran, Ketawanggede, Lowokwaru, Malang 65145, East Java
*Corresponding email: nnbarunawati.fp@ub.ac.id
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ABSTRACT

Celery is a plant that produces secondary metabolites, known as flavonoids, with distinctive taste and smell. Flavonoids are synthesized in leaves and mainly accumulate in vacuoles of cells. Water deficit is one of the abiotic stress factors that affect the increase of several amino acids as cofactors that form secondary metabolites. Moreover, nitrogen nutrients have an essential role in the formation of amino acids and chlorophyll. The research results show that the total fresh weight increased at around 450 g by applying 200 kg N ha^{-1} under 100% field capacity (FC). Consequently, there was an increase in fresh weight, followed by the biomass of celery, which was 1/15 of fresh weight. Meanwhile, the accumulated flavonoid concentration was higher at 50% field capacity and 100 kg N ha^{-1}. Surprisingly, the content of proline and flavonoids in leaves of the same age had higher concentrations at 100 kg N ha^{-1} and 50% FC compared to the concentration of proline and flavonoids at 200 kg N ha^{-1} in the form of urea fertilizer, namely 0.25 µmol g^{-1} and 67.84 ppm, respectively. At 50% FC and 100 kg N ha^{-1}, it is slightly higher on proline and flavonoids than the application of 100% FC and 200 kg N ha^{-1}. The highest accumulation of phosphorus in leaves is interestingly at around 0.51 ppm at 50% FC, compared to the application of 100% FC at 100 and 200 kg N ha^{-1} half bellows at around 0.3.

Keywords: celery, flavonoids, nitrogen, proline, water deficit

ABSTRAK

Seledri merupakan tanaman yang menghasilkan metabolit sekunder yang dikenal sebagai flavonoid, dengan rasa dan bau yang khas. Flavonoid disintesis di daun dan terutama terakumulasi di vakuola sel. Defisit air merupakan salah satu faktor cekaman abiotik yang mempengaruhi peningkatan beberapa asam amino sebagai kofaktor pembentuk metabolit sekunder. Selain itu, nutrisi nitrogen memiliki peran penting dalam pembentukan asam amino dan klorofil. Hasil penelitian menunjukkan bahwa bobot segar total meningkat sekitar 450 g dengan pemberian 200 kg N ha-1 pada kapasitas lapang (FC) 100%. Akibatnya terjadi peningkatan bobot segar yang diikuti dengan biomassa seledri sebesar 1/15 bobot segar. Sedangkan konsentrasi akumulasi flavonoid lebih tinggi pada kapasitas lapang 50% dan 100 kg N ha-1. Yang mengejutkan, kandungan prolin dan flavonoid pada daun berumur sama mempunyai konsentrasi lebih tinggi pada 100 kg N ha-1 dan 50% FC dibandingkan dengan konsentrasi prolin dan flavonoid pada 200 kg N ha-1 dalam bentuk pupuk urea, yaitu masing-masing 0,25 µmol g-1 dan 67,84 ppm. Pada 50% FC dan 100 kg N ha-1, prolin dan flavonoidnya sedikit lebih tinggi dibandingkan aplikasi 100% FC dan 200 kg N ha-1. Menariknya, akumulasi fosfor tertinggi pada daun adalah sekitar 0,51 ppm pada 50% FC, dibandingkan dengan penerapan 100% FC pada 100 dan 200 kg N ha-1 half bellow sekitar 0,3.

Kata kunci: seledri, flavonoid, nitrogen, prolin, defisit air

1. INTRODUCTION

Nitrogen is a macronutrient essential in forming amino acids, nucleic acids, and chlorophyll for plant growth and development (Shrivastav et al., 2020). The nutrient element nitrogen triggers the vegetative growth of plants, which affects leaf colour, leaf length, leaf width, and length of plant stem growth (Leghari et al., 2016). Studies previously by Mu et al., (2016) show that nitrogen can help increase results and enhance the efficiency of photosynthesis in plants. Febrianna et al., (2018) report that the absorption of Nitrogen elements in vegetable plants can seen from enhanced weight drying of the plant. In onion plants, leaf enhancement Nitrogen nutrients can increase the fresh weight of plants (Gonçalves et al., 2019). Nitrogen is absorbed by plants as NO_3 and NH^{4+} (Krapp, 2015). Nitrogen is accumulated in the plant as nitrate (NO_3) (Ravazzolo et al., 2020).

Water is part of the main plant, which forms 80-90% of the tissue in the plant in the stage of development. Based on Filipović (2020), water plays a crucial role in facilitating the growth of plants by supporting fundamental metabolic functions and meeting essential hydration requirements during critical stages of their development. Lack of water in plants will lower the rate of division and elongation of cells (Qi & Zhang, 2020). Apart from that, plants will also lack water, which reduces nutrient absorption in the soil, photosynthesis, development of plant tissues, and metabolism (Majláth et al., 2016). The role of water as a solvent for soil nutrients causes plants to grow by absorbing nutrients in the soil through roots and transporting nutrients to some plant organs. Lack of water in plants will disrupt activity physiologically and morphologically, influencing plant size and intensity in both vegetative and generative phases. Based on the study by Song et al., (2019), water stress can reduce growth and decline in plant fresh weight. According to Merwad et al., (2018), water supply influences plants' leave numbers, fresh weight, and dry weight.

Abrar et al., (2022) state that drought stress in celery plants affects plant height of 28.4 cm, and the root fresh weight is 6.6 g. Grip dryness in the plant indicates responses like the closing of the stomata, which is a decline in the number and the area of plant leaves. According to Roig-Oliver et al., (2021), a lack of water in plant tissue can reduce cell turgor and water activity in plants; water shortages will occur in plants, ultimately influencing plant metabolic processes and growth and development. A dose of nitrogen fertilizer and a proper water supply for celery plants improve plant growth and avoid the condition of the plant's lack of water, which can reduce the absorption of nutrients in plants. This research aims to discover interactions between different doses of nitrogen fertilizer and supplying different amounts of water (different FC).

2. METHODOLOGY

The research was conducted as a field trial from May to July 2022 in Batu, East Java, Indonesia. The research area is located at an altitude of 910 meters above sea level with an average temperature of 19-22 °C in a plastic house. This study was a factorial experiment with a randomized block design (RBD) with 2 factors and 3 replications. The first factor is the dose of urea fertilizer, which consists of 2 levels, namely 100 N: Nitrogen 100 kg ha^{-1} and 200 N = Nitrogen 200 kg ha^{-1}. The second factor is the volume of water supply, which consists of 2 levels, including 50% = 790 ml (50% Field Capacity) and 100% = 1580 ml (Field Capacity 100 %). To determine the field capacity, it is done by giving water to the polybag every 3 days according to the water supply's levels, provided the soil in the polybag is dry. Each treatment has four destructive samples, which are used to measure fresh weight, dry weight, nitrogen, phosphorus, proline, and flavonoid content. Protocol observation of proline in fresh leaves measured with a spectrophotometer. Whereas the observation of nitrogen was determined from flag leaves using ES-MS (EA: Elemental Analyzer, Mass Spectrometry) (Barunawati et al., 2019). Phosphorus content in leaves was assessed using the ash technique using a combination of $HClO_4$ and HNO_3 (Kailola et al., 2023). Further analysis in celery leaves was total flavonoid content determined using a protocol according to Ahmed et al., (2015).

3. RESULT AND DISCUSSION

3.1 Fresh Weight

We found that the dose of urea fertilizer and water supply increases the plant's fresh weight by 25%. The treatment of 100% water in 100 and 200 kg N ha^{-1} applications shows lower results than 50% water supply. Thus, the different levels of water supply and nitrogen application affect the fresh weight of the celery plants. The average plant fresh weight at 12 weeks after planting (WAP) is presented in Figure 1. According to Qi et al., (2020), nitrogen supply and irrigation management increase root growth, resulting in increased water use efficiency and plant biomass in maize. Increasing nitrogen fertilizer under high water availability also increases plants' antioxidant and photosynthetic rates (Tian et al., 2020).

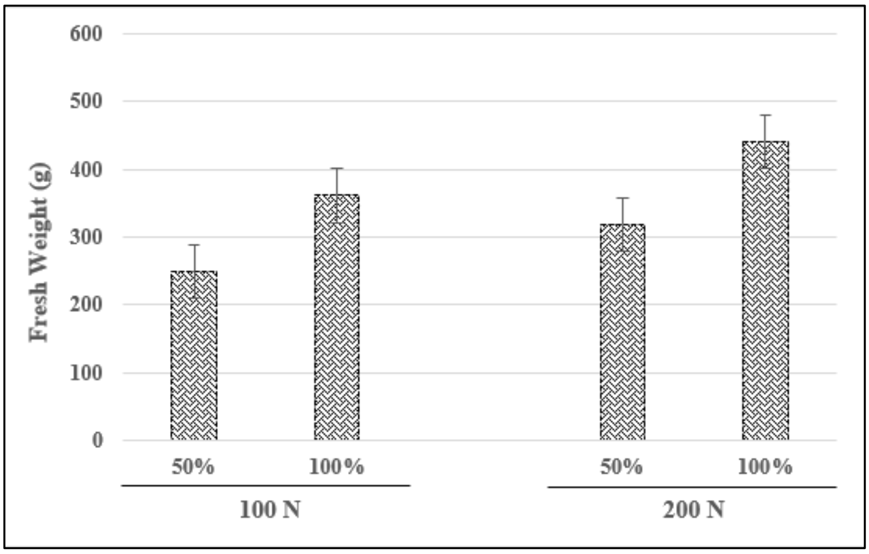

Figure 1. Effect of nitrogen (N) application on water supply on fresh weight of celery at 12 WAP

3.2 Dry Weight

The application of nitrogen fertilizer and water supply results in an increase in plant biomass. On the other hand, when the water availability is 50%, and the nitrogen supply is low, it tends to lower celery plant biomass. However, increasing the dose of N fertilizer up to 200 kg N ha^{-1} followed by increasing plants' dry weight under 50% and 100% water supply. Figure 2 shows the dry weight of celery plants at 12 WAP. Trend graph plant biomass tends to be equal to chart weight fresh plants. A higher nitrogen fertilizer dosage accelerates dry matter accumulation rate and time simultaneously, which impacts plant production (Zhai et al., 2022). In contrast, the reduction of water supplies reduced plant growth parameters, including plant height, leaf number, and dry weight (Luo et al., 2020). Fresh and dry weight of celery plant shows a graphic trend that tends to be the same, namely at 50% water supply, and application 100N kg ha^{-1} shows lower results by 36%. Meanwhile, at 100% FC and application, 100 kg N ha-1 shows higher results compared to 50% FC. This can be done assuming that The increased biomass (64 %) was influenced by adding nitrogen fertilizer 200 kg ha^{-1}. Moreover, increasing nitrogen fertilizer is also followed by increased plant fresh weight and biomass of celery plants.

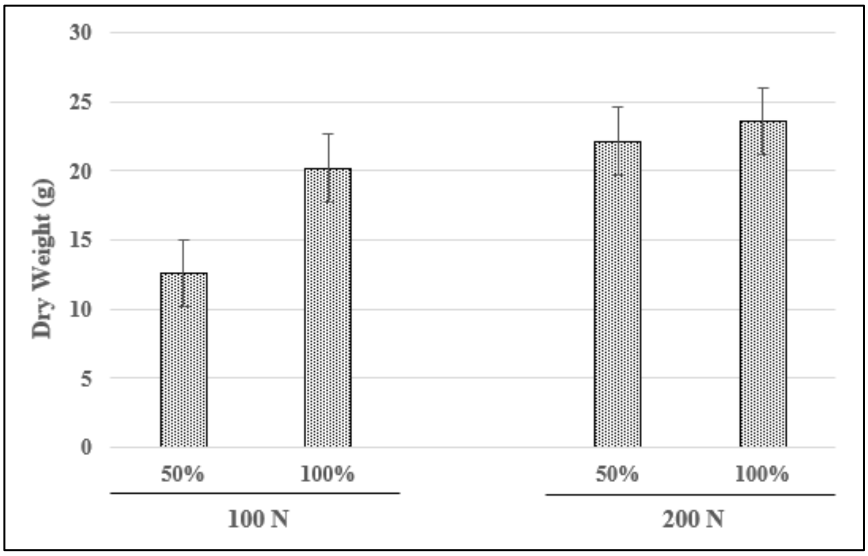

Figure 2. Effect of nitrogen application on water supply on dry weight of celery at 12 WAP

3.3 Concentration of Nitrogen

Our results showed that the treatment of different water supply levels and N fertilizer gave a similar result on nitrogen concentration in the leaves. In general, 50% and 100% water supply and 100 and 200 kg N ha^{-1} show that leaf nitrogen concentrations are the same. Concentration in celery plants can seen in Figure 3. Drought stress increases plant transpiration and limits nitrogen absorption. This can be interpreted that nitrogen and water play an important role in plant metabolism. Water and nitrogen availability have an interactive impact that results in nitrogen metabolism, affecting physiological and biochemical changes significant for drought tolerance (Ding et al., 2018). However, the current study shows that nitrogen concentration in leaves tends to be the same for every treatment. In addition, applying N fertilizer improved plant tolerance to drought stress by maintaining water content, water use efficiency, and antioxidant enzymes (Agami et al., 2018). This can be done assuming that medium and high nitrogen supply increases plant resistance to drought. At the same time, stable nitrogen concentration shows plant sensitivity to stress drought.

Based on the results of a study previously by Xiong et al., (2018), it found an impact that has a significant interaction between nitrogen and water stress content nitrate and leaf ammonium. Application of a high nitrogen dose with the stressed condition clearly affects the nitrogen content in leaves. Under high nitrogen and water stress change conditions, nitrogen allocation becomes proline or other nitrogen compounds (Chechin et al., 2022). Based on the results, studies show that nitrogen concentrations tend to be the same in all treatments (Figure 3).

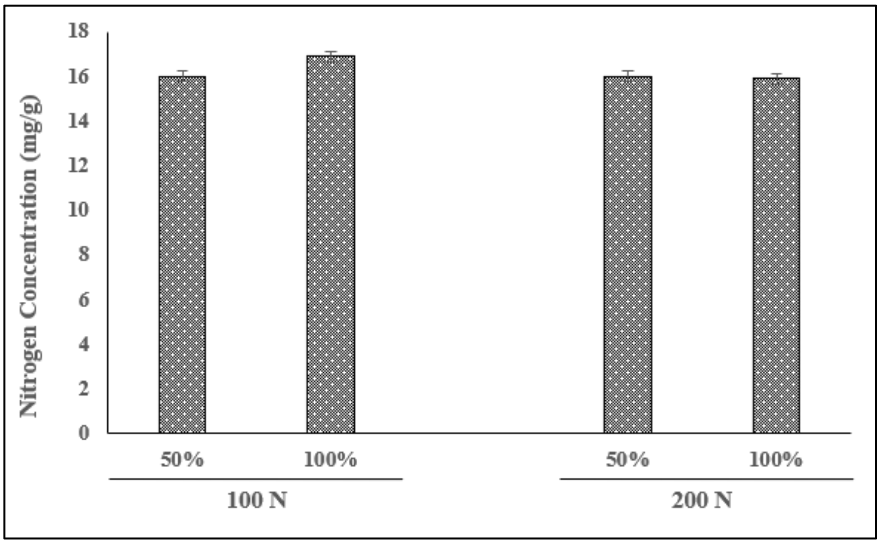

Figure 3. Effect of nitrogen (N) fertilizer on water supply on leaves nitrogen concentration

3.4 Concentration of Phosphorus

Our result revealed that phosphorus concentration in celery leaves tends to decrease the consequence of adding nitrogen. Treatment supplying 50% water and urea application of 100 kg N ha^{-1} increases the concentration of phosphorus in leaves celery by 64% compared to supplying 100% water. In the trend graph, phosphor tends to decrease by 100% in water treatment and by 200 kg N ha^{-1} in urea treatment. Meanwhile, 200 kg N ha^{-1} urea treatment with 100% water applied shows lower phosphorus concentration than other treatments. The concentration of phosphorus in celery leaves is shown in Figure 4. Based on the results, the study shows that phosphorus concentration in the leaves is higher up to 54% in the 50% FC and 100 kg N ha^{-1} treatments compared to 50% FC and 200 kg N ha^{-1}, to the results of a study previously conducted by Kuwahara et al. (2016), who stated that on the condition of lack of water, plants tend to develop plant roots deeper to get water. Hence, a mechanical process transports P from the soil through diffusion flux. On condition dryness, pattern rooting influences soil absorption from water and phosphorus.

Based on Getachew's statement (2014), the trend is on the layer under normal phosphate conditions. The topmost plant is the most vulnerable to drought, so absorption becomes easier, although the patterns are shallow roots. However, depending on conditions of dryness, the pattern of deeper roots tends to increase the absorption of water and P in the soil (Yuan et al., 2016). Land with conditions Lack of water results in low cell turgor, and plant metabolic processes are hampered. Decreasing the concentration of phosphorus in plants increases proline content in leaf tissue as a regulator of osmotic pressure in plants (Aleksza et al., 2018).

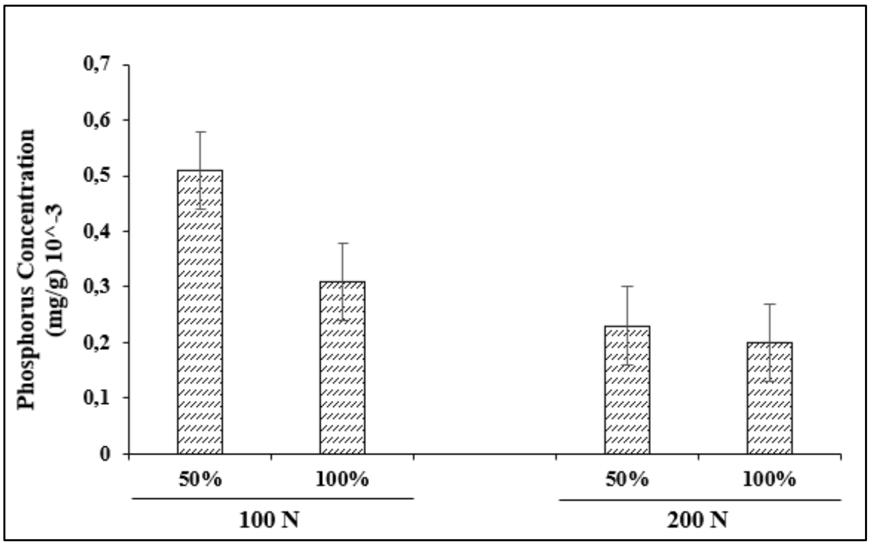

Figure 4. Effect of nitrogen (N) fertilizer on water supply on leaves phosphorus concentration

3.5 Concentration of Proline

The concentration of proline is presented in Figure 5. Overall, our results exhibited that supplying water by 50% and applying 100 kg N ha^{-1} urea increased proline content by 0.5% compared to other treatments. Meanwhile, in treatment with 100% water and 100 kg N ha^{-1}, proline concentrations tend to be the same as in treatment with 200 kg N ha^{-1} at capacity, applying 50% and 100% water. Proline is formed as a plant defense compound, and the consequence exists under drought stress. Plants with a condition that lacks water (water deficit) can synthesize and accumulate several osmolytes in cells to reduce plant osmotic potential. Synthesis and accumulation of osmolytes include amino acids like proline, sugar, and polyamine (Ghosh et al., 2021). Accumulation from proline increases assimilation from CO_2 and protects cell membranes. Proline is linked with tolerance to stress on plants. Molla et al., (2021) stated that accumulation from higher proline assumed can help better osmotic maintenance under water deficit.

Proline (Pro) is an amino acid that has a role in plant tolerance to stressful environments. This is related to preventing protein oxidation, reducing lipid peroxidation, and maintaining cell membranes and structures. The proline is a nitrogen and energy source (Liu et al., 2017). Proline protects proteins and chlorophyll from degradation cells and improves some enzyme activities (Alhaithloul, 2019). Amino acids are primary metabolites produced by plants that play an essential role in the biosynthesis of proteins, enzymes, and nitrogen-containing molecules for plant development and defense. According to Chechin et al., (2022), proline is an amino acid containing nitrogen. Synthesis increased proline affected by high nitrogen supply. However, this differs from the results of research conducted that concentration proline with higher nitrogen applications at 200 kg N ha^{-1} treatment tends to be the same at 50% and 100% water supply.

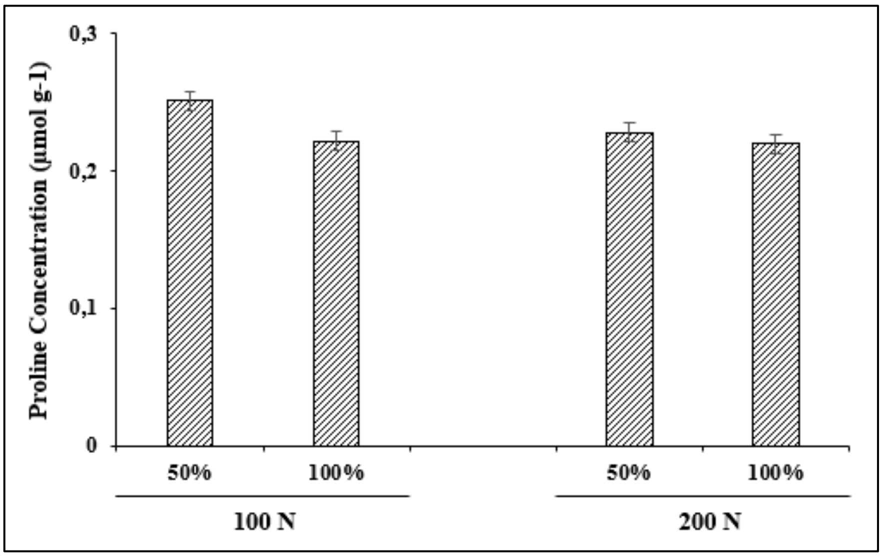

Figure 5. Effect of nitrogen (N) fertilizer on water supply on leaves proline concentration

3.6 Concentration of Flavonoids

Our results showed that the concentration of flavonoids in celery in the treatments supplying 50% water at 100 kg N ha^{-1} shows higher flavonoid concentration by 18% compared to other treatments. Meanwhile, applying 50% water and 100 kg N ha^{-1} shows that flavonoid content tends to be the same as treatment 200 kg N ha^{-1}, which is better at 50% water supply. The concentration of flavonoids in celery plant flavonoids at 12 WAP is shown in Figure 6. Flavonoids are metabolites of polyphenols with heavy molecules in abundance, and they have different biological activities in plants (Shomali et al., 2022). Based on location and quantity group, the hydroxyls of flavonoids are grouped into flavonols, flavones, isoflavones, flavanones, flavonols, and anthocyanidins (Khalid et al., 2019). Plants synthesize flavonoids through track phenylpropanoid (Liu et al., 2021). Flavonoids contribute to the plant's response to abiotic stress and their role in differentiating cells, growth, and signalling defense (Samec et al., 2021).

The results show that nitrogen and water applications significantly affect the variables observed in Flavonoid concentration in leaves. The results showed that flavonoid concentrations increased by 2% in 50% water and 100 kg N ha^{-1}. According to Ma et al., (2014), the rate of flavonoids in leaves of *Triticum aestivum* increases when the plant is subjected to drought stress. Supported by Zhao et al., (2016), drought can also increase the expression of several flavonoid biosynthetic genes in the roots of *Scutellaria baicalensis.*

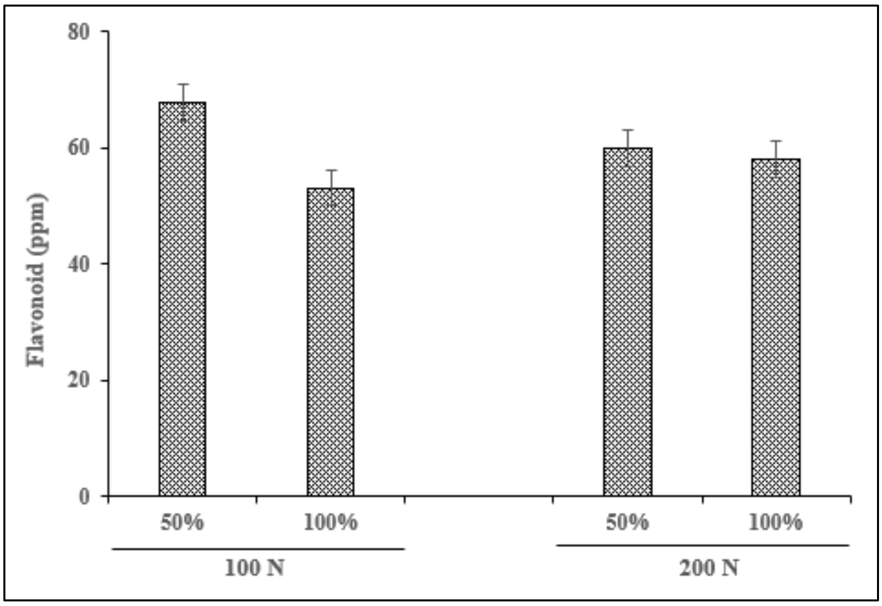

Figure 6. Effect of nitrogen (N) fertilizer on water supply on leaves flavonoid concentration

4. CONCLUSION

Under conditions of lower N and lack of water, the accumulation of proline and flavonoids was higher under conditions of insufficient water supply and nitrogen availability, namely 0.25 µmol proline g^{-1} and 67.84 ppm, respectively. The results indicate that proline and flavonoid are osmoprotectants critical in plant defense against abiotic stress, mainly water deficit.

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