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The Nitrogen Effect on Plant Growth and Protein Production in Maize

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Abstract | Nitrogen is an essential element for maize growth. The crude protein produced by maize is related to the amount of nitrogen within the tissue. Different amounts of nitrogen could generate different amounts of crude proteins in maize plants. This study focuses on the determination of the crude protein production from different amounts of nitrogen composition in the fertilizers. A total of sixty maize seeds (GWG 888) were planted at Universiti Sultan Zainal Abidin (UniSZA) Kampus Besut Farm. Four different fertilizer treatments were used for the plant species, including control, NovaTec, foliar, and urea. The data for morphological and physiological characteristics, including plant height, the distance of nodes, leaf width, and leaf length, were taken every two weeks. The physiological parameters, including chlorophyll content and stomatal conductance, were recorded during plant growth. The results showed that there is an increase in crude protein content from the difference in nitrogen fertilizer treatment compared to control. The NovaTec fertilizer treatment produces the highest crude protein in the kernel and leaf in maize, followed by urea and foliar. It can be concluded that a higher amount of nitrogen can induce higher production of crude protein in maize.

Received | February 22, 2024; **Accepted** | August 06, 2024; **Published** | October 07, 2024***Correspondence** | Mohd Fahmi Abu Bakar, School of Agricultural Sciences and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut, Malaysia; **Email:** mohdfahmi@unisza.edu.my**Citation** | Romzi, N.M.I., A.Z.M. Suhaili, N. Ngah and M.F.A. Bakar. 2024. The nitrogen effect on plant growth and protein production in maize. *Sarhad Journal of Agriculture*, 40(Special issue 1): 50-60.**DOI** | <https://dx.doi.org/10.17582/journal.sja/2024/40/s1.50.60>**Keywords** | Crude protein, Fertilizer, Maize, Morphology, Nitrogen**Copyright:** 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Introduction

Maize (*Zea mays* L.) belongs to the family of grasses (Poaceae), the panicle subfamily of Panicoideae, and the tribe Andropogoneae. It is also known as corn (Adhikari *et al.*, 2021). It is widely grown in the Americas, Europe, Africa, and Asia (Fujisao *et al.*, 2020). Maize has become a staple food in Africa, Latin America, and Asia, as well as a commodity in industrialized nations (Santproort,

2020). There are many different types of maize depending on the shape of the kernel and composition of the endosperm (Awata *et al.*, 2019). The common types of maize include flint, dent, floury and waxy, pop, and sweet corn (Nor *et al.*, 2019). Apart from that, there are also many different kernel colours, including yellow, white, red, blue, pink, and black types that are often banded, speckled, or striped, where the former is the most commonly used as a food (Britannica, 2023).

Maize is an important food as a cereal for humans and animals, including ruminants and poultry (Abiodun *et al.*, 2021). According to the worldwide grain production in 2022/2023, maize has become the third most important cereal crop in the world, after wheat and rice (Shahbandeh, 2024). The annual production exceeds 1 billion tonnes (Garca-Lara and Serna-Saldivar, 2019). Most of the maize has been used as livestock feed, while humans consume small portions (Shiferaw *et al.*, 2011). Maize offers important sources of vitamins and minerals to humans as well as energy, oil, starch, biofuel, and protein sources for ruminants and poultries (Shiferaw *et al.*, 2011). The livestock can be fed with maize grits, bran, germ, and oil meal (Kaul *et al.*, 2019). The livestock diet needs carbohydrates and protein as major nutrients for their growth. Currently, maize has high carbohydrates with low protein content. Protein is an extremely complex nitrogen-containing organic molecule that serves as the foundation for life (Westerterp-Plantenga, 2003). Livestock needs several important amino acids in the diet, such as lysine, tryptophan, and methionine, for their growth (Samtiya, 2020). The majority of animal protein needs are obtained through the consumption of plant protein (Huang *et al.*, 2022). The production of amino acids is related to the nitrogen consumption by the maize. Since the animal feed price increases almost every month, there is a need to produce maize varieties with high protein biosynthesis, as it is a very important diet in animal feed. Malaysia has imported almost 93% of the total maize for animal feed production, and millions of tons of maize are needed to meet the demand (Rosali, 2019).

Maize contributes to global food security by providing 20% of calories and 15% of protein (Liu *et al.*, 2020). Maize is more productive than wheat in terms of nutrients provided (Liu *et al.*, 2020). The germ, pericarp, and endosperm make up a mature corn kernel. The endosperm contains 90% of starch, a concentrated energy source, and 10% of protein, with zein protein accounting for 50-70% of the total protein (Krishna, 2020). The relative proportions of different protein fractions and their amino acid compositions determine the maize kernel amino acid composition (Dei, 2017). Other parts of the maize kernel that have high-quality protein become recessive because zein dominates in normal maize (Shawa *et al.*, 2021).

Protein is the second-largest chemical constituent after starch within the kernel. The protein content is

between 8% and 11% of the kernel weight, where the endosperm contains most of it. According to Landry and Moureaux (1982), maize is composed of at least five distinct fractions where albumins, globulins, and non-protein nitrogen make up a total of 18% of the total nitrogen, with distributions of 7%, 5%, and 6%, respectively. However, recent studies proposed that albumins (3%), globulins (3%), and glutelins (34%) contribute between 30-40% of the total protein (Sethi *et al.*, 2021). Moreover, zein, the major prolamin fraction, forms the bulk of commercially available corn protein, reaching over 90% purity and showcasing film-forming abilities similar to wheat gluten. These hydrophobic proteins make up about 60% of maize protein and are primarily found in the endosperm (FAO, 2005). While they're relatively low in essential amino acids, zeins possess unique film-forming properties, making them valuable in industrial applications. On the other hand, glutelins and sulfur-rich proteins constitute roughly 25% of maize protein and contribute to its gluten content (FAO, 2005). They play a crucial role in seed structure and contribute to the nutritional value of maize for both humans and livestock.

Nitrogen is a very important macronutrient for plant growth (Mustapha *et al.*, 2018). Nitrogen is involved in the production of protoplasm and amino acids for tissue and protein formation, as well as enzymes that support biochemical processes (No-Atirah *et al.*, 2023). Nitrogen uptake by plant roots is in the form of nitrate, ammonium, and anhydrite ions from the soil (Ismail *et al.*, 2016). In the cytosol, nitrate is changed into nitrite, which is then changed into ammonium in the plastids (Ward *et al.*, 2018). The conversion process can happen in the roots or through the xylem in the leaves (Yoneyama and Suzuki, 2019). The optimum usage of nitrogen has been reported to increase photosynthetic processes, leaf area production, and net assimilation rate (Leghari *et al.*, 2016). According to Marschner (2012), nitrogen is an essential macronutrient used for plant growth and development, promoting rapid cell division and expansion, resulting in larger leaves and increased biomass.

Previous studies show that improving amino acid production can increase nutritional value as well as reduce protein deficiency problems in humans and animals (Maqbool *et al.*, 2021). A total of 22 hybrid maizes developed by Padjadjaran University have

successfully increased protein content compared to inbred maize (Indriani *et al.*, 2018). Other studies have been reported on protein production related to nitrogen consumption by plants, such as in buckwheat (Wan *et al.*, 2023) and Welsh onion (Zhao *et al.*, 2021).

Since there is less studies related to the effect of nitrogen on protein production in maize, there is a need to understand the physiological and morphological changes of maize toward different nitrogen treatments. This study focuses on the effect of nitrogen on plant growth and protein production in maize. We hypothesized that the nitrogen within fertilizer could increase the crude protein in maize. This study aims to identify the changes in the morphological and physiological characteristics of different fertilizers and determine the relationship between the different nitrogen content and protein production in maize.

Materials and Methods

Planting method

Maize grows best on well-drained, well-aerated, deep soils with enough organic matter and nutrients (Chibuzor, 2021). In this study, maize was grown on the Beach Ridges Interspersed with Swales (BRIS) soil. The maize seeds (GWG 888) were put in the tray for seven to ten days and covered with black plastic to germinate in the tray. The seed was then transplanted to the field at a depth of three to five centimetres in rows, with 20–24 centimetres between seeds and 60–68 centimetres between rows. The NPK Green (15:15:15) fertilizer was given to each plant after 7 days after transplanting. Moreover, NPK Blue fertilizer (12:12:17) was given to each plant after 21 days to continue delivering nutrients to the maize. There were three distinct types of fertilizers (Control, NovaTec, Foliar, and Urea), with fifteen replicates of each treatment. After 30 days, the maize plants subsequently received the fertilizer treatments, excluding control. A total of five grams was given to each plant. This is because, for the plant to thrive and produce at its highest levels, it has to receive enough nutrients (Sugiono *et al.*, 2020). Watering is done in accordance with the plant and soil conditions (Xu *et al.*, 2010). The total water demand for maize ranges from 530 to 800 millimetres (Hiranmayee, 2020).

Measurement of maize morphological parameters

Height of maize plant: The measurements were done from planting until the harvesting process. The height of the plant was measured from the plant's base at the soil's surface to the highest point of the downward-pointing arch of the leaf at its end. A measuring tape was used as the instrument.

The distance of the node: The parameter was calculated to observe changes between nodes in each treatment and record all the data for each plant. The measurement was taken every two weeks. The node was measured by using a measuring tape (Liu *et al.*, 2020).

Leaf width: The leaf width was measured on the leaf at the widest part. The parameter was calculated once every two weeks until harvesting. This parameter was measured with measuring tape. Leaf width was closely associated with specific leaf weight (Liu *et al.*, 2020).

Leaf length: The leaf length was measured at the widest point of the leaf. The parameter was calculated once every two weeks until harvesting. This parameter was measured with measuring tape. Measure from the top of the entire leaf maize to the bottom of the leaflets (Mokhtarpour *et al.*, 2010).

Measurement of maize physiological parameters

Chlorophyll content: The chlorophyll content showed the amount of photosynthesis in the leaf. The chlorophyll content was read using Chlorophyll Meter SPAD-502 (SPAD502 Minolta, Japan). The reading shows the index of chlorophyll a and b in the thylakoid membrane. The reading was measured early in the morning to determine the amount of chlorophyll in the leaf accurately (Kandel, 2020).

Stomatal conductance: The portable leaf porometer was used to measure stomatal conductance. A leaf chamber was attached to the leaf and kept at an ambient temperature for 10 to 15 minutes to maintain sunlight adaption. The stomatal conductance is an important indicator of crop water stress and moisture content during the entire phenological cycle (Brewer, 2022).

Protein determination: The specimens were ground using a cereal mill. A total of two grams of specimen was transferred in a Kjeldahl test tube. One tablet of catalyst Kjeldahl and the chemical solution was added into the tube (Thiex *et al.*, 2003). Blanks with all

chemicals without any samples in the test tube were then prepared. In the digesting block, the samples were heated at 180°C for 75 minutes, followed by 320°C for 4 to 5 hours, until the sample became colourless. The distillate was collected in an indicator solution containing 2% boric acid. During distillation, the colour changes from purple to green. The boric acid was then boiled and stirred until dissolved. The mixture was then chilled. The mixture was titrated against 0.01 M HCl until the green colour changed to purple. The data was recorded (Thiex *et al.*, 2003).

Statistical analysis

The randomized complete block design (RCBD), an experimental design was used in this study with fifteen replications. The data obtained from two successive seasons were pooled and analysed using Microsoft Excel. A one-way ANOVA was used to analyze and evaluate the differences in the studied parameters in the different treatments. The least significant difference (Fisher's protected LSD) was calculated following a significance *F*-test (at $P=0.05$).

Results and Discussion

Morphological parameters

The morphological characteristics that have been recorded include the plant height, distance of nodes, leaf length, and leaf width of maize. According to the data, there is a significant difference in the morphological characteristics of different fertilizer treatments. The plant height character is one of the useful indicators for the growth and development of maize since it is genetically determined (Peiffer *et al.*, 2014). The height characteristic serves as a reliable indicator of overall plant health and vigor (Zhang *et al.*, 2022). Taller plants generally translate to greater biomass production, crucial for both forage and grain yield (Borras *et al.*, 2003). Furthermore, plant height is strongly correlated with grain yield, making it a valuable tool for predicting crop performance (Bänziger *et al.*, 2006). Taller plants generally have more leaves for photosynthesis, leading to increased grain production. Studies by Borrell *et al.* (2005) found that a 10 cm increase in maize height could translate to a 5% yield increase. The results showed that the difference in the amount of nitrogen given has affected the maize height (Figure 1). The NovaTec fertilizers-treated maize produced the tallest plant from week 2 until week 20. Generally, maize can grow between 40cm and 100cm. According to Xiangbei *et*

al. (2022), nitrogen application helps maize cropping generate high yields and sustainable agricultural development. The increase in the nitrogen supply can also improve maize growth (Olowoboko *et al.*, 2017). On the other hand, nitrogen application has been found to increase mesophyll cell division and epidermal cell elongation in *Festuca arundinaceae* Schreb (MacAdam *et al.*, 1989). Thus, the addition of NovaTec fertilizer has increased the plant height compared to other treatments.

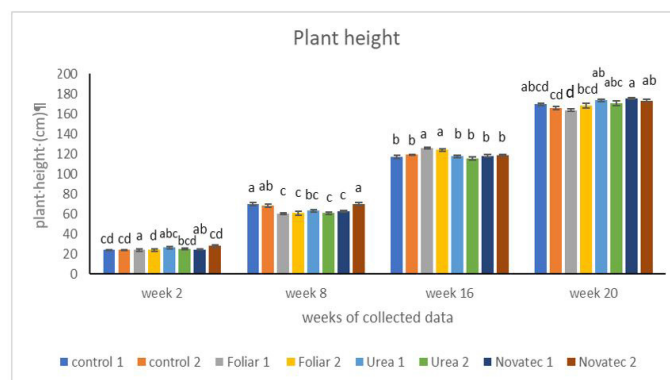


Figure 1: The effect of different fertilizers on plant height of maize seedlings from the 2nd week to the 20th week. Means followed by the same lowercase letters within the same coloured column are not significantly different according to tests LSD.

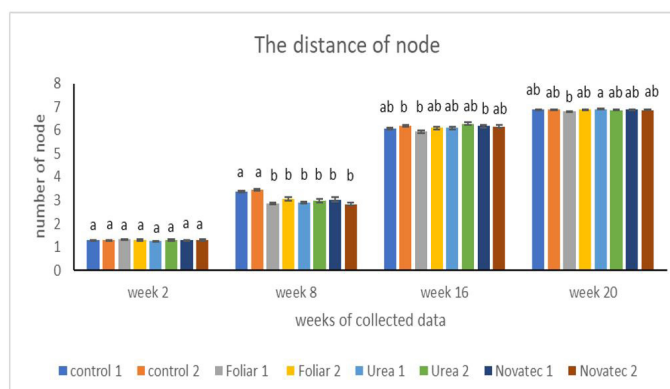


Figure 2: The effect of different fertilizers on node distance of maize seedlings from the 2nd week to the 20th week. Means followed by the same lowercase letters within the same coloured column are not significantly different according to tests LSD.

Apart from that, the node distance, which is also known as internodes, is dependent on the species or variety itself. Since only a maize variety was used in the study, the internode size should be the same. Interactions between root nodes have significant effects on crop nutrient uptake and tolerance to stress. According to the data collection, the distance between nodes on a maize plant was nearly identical for different fertilizer treatments at week 20 (Figure 2). Although at week 8, the internode size differed, as the plant grew, the node distance was nearly the same.

The average growth angles of node distance maize were 7 cm (Schneider *et al.*, 2021). However, the internode size can be higher in the increased nitrogen conditions. The high amount of nitrogen fertilizer can increase the number of leaves due to the increase in the number of nodes (Amin *et al.*, 2011).

The result showed that the leaf had almost the same length and width for each treatment from week 2 until week 20 (Figures 3 and 4). Our results are supported by the findings of Olowoboko *et al.* (2017), who stated that the leaf length and leaf width do not depend on the amount of nitrogen in maize. The leaf width did not significantly differ following the application of nitrogen fertilizer (Luo *et al.*, 2021).

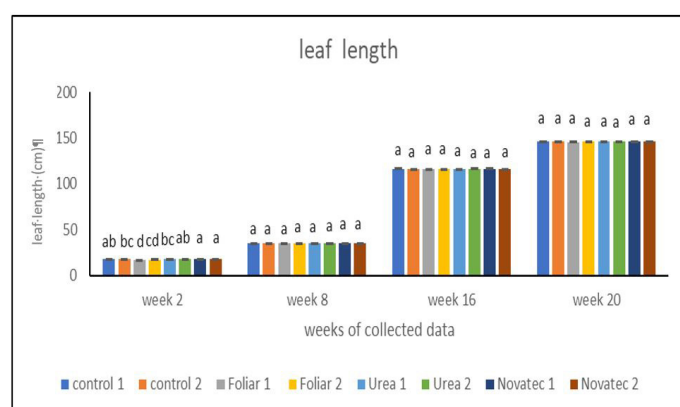


Figure 3: The effect of different fertilizers on the leaf length of maize seedlings from the 2nd week to the 20th week. Means followed by the same lowercase letters within the same coloured column are not significantly different according to tests LSD.

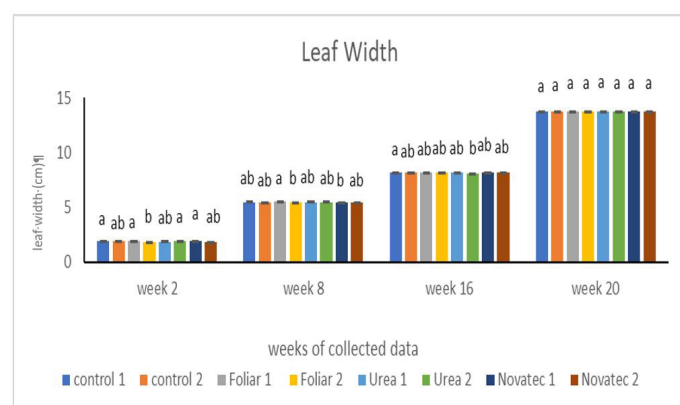


Figure 4: The effect of different fertilizers on the leaf width of maize seedlings from the 2nd week to the 20th week. Means followed by the same lowercase letters within the same coloured column are not significantly different according to tests LSD.

Physiological parameters

The physiological parameters that have been recorded include the chlorophyll content and stomatal conductance. The results showed that fertilizer treatments produced significant effects on

the improvement of the physiological parameters of maize. The chlorophyll content has a relationship with the photosynthesis process, which is important for the growth and development of maize. The result indicated that the NovaTec fertilizers-treated maize maintained the highest chlorophyll content from week 2 until week 18 (Figure 5). According to Pandey *et al.* (2020), an average of 45 mmol/cm² chlorophyll was recorded from SPAD meter readings of the maize's leaf. The results showed that the NovaTec fertilizer-treated maize had between 40 and 50 mmol/cm² of chlorophyll from week 2 until week 18. The results indicated that nitrogen can affect chlorophyll synthesis in maize. This result is also supported by the findings of Tsai and Chang (2020), who reported that nitrogen increases the concentration of chlorophyll in plants.

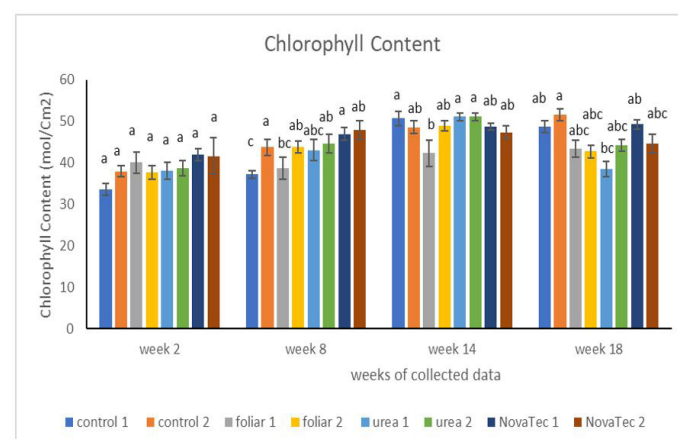


Figure 5: The effect of different fertilizers on chlorophyll content of maize seedlings from the 2nd week to the 18th week. Means followed by the same lowercase letters within the same colored column are not significantly different according to tests LSD.

A stomatal conductance analysis was done to estimate the rate of gaseous exchange and transpiration through the leaf stomata (Gong *et al.*, 2023). The higher the stomatal conductance, the higher the photosynthesis and transpiration rate. The result showed that the NovaTec fertilizers-treated maize maintained the highest stomatal conductance from week 2 until week 18, while other fertilizer treatments had rapid changes in the parameter (Figure 6). The results were also supported by a study done by Khamis *et al.* (2020), which found the photosynthetic rate is directly proportional to stomatal conductance. Apart from that, Liao *et al.* (2022) reported that the stomatal conductance was affected by drought and nitrogen stress. The result of this study indicated that the difference in the amount of nitrogen given can induce the opening of stomata and accelerate the

photosynthesis and transpiration process.

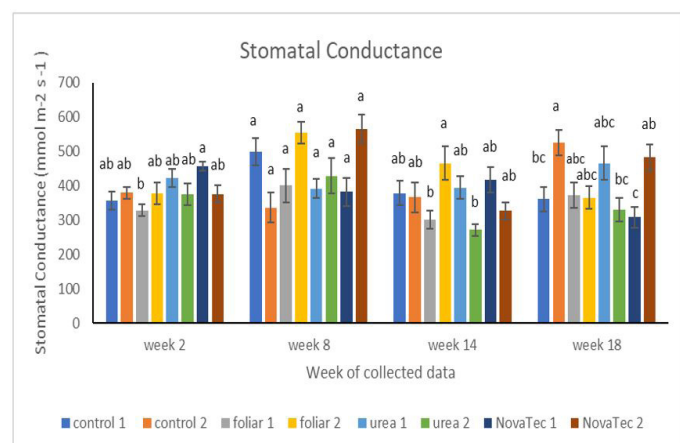


Figure 6: The effect of different fertilizers on stomatal conductance of maize seedlings from the 2nd week to the 18th week. Means followed by the same lowercase letters within the same colored column are significantly different according to tests LSD.

Protein analysis

The Randomised Complete Block Design (RCBD) was utilized to set up three distinct types of fertilizers (Control, NovaTec, Foliar, and Urea), with fifteen replicates of each treatment. The RCBD was used due to its versatile design, which is suitable for various agricultural experiments, from comparing different crop varieties to testing the effectiveness of new fertilizers or pest control methods (Gomez and Gomez, 2015). The crude protein analysis shows the amount of protein produced from kernel and leaf specimens. The results showed that the NovaTec fertilizers-treated kernel has higher protein production compared to others (Figure 7). The high protein content in NovaTec fertilizer-treated kernel may be due to the fertilizer application at the basal of the maize plant. The efficiency of nutrient transportation from the root as a source to the kernel as a sink is dependent on the efficiency of translocation of ion uptake (Novak and Vidovic, 2003). Although urea has a higher amount of nitrogen, the NovaTec fertilizers, which have a balanced NPK ratio, have induced a high protein amount. According to Wang *et al.* (2023), the application of good fertilizer at the basal of rice plants has improved the grain quality and protein amount, while a higher amount of N fertilizer ratio application at the basal can decrease the protein production.

Moreover, there is slightly lower protein production of maize kernel 2 than kernel 1 (Figure 7). The higher the number of cob in maize, the lower the protein amount within the kernel. The result is also

supported by Liu *et al.* (2018), who stated that the nutrient distribution within the plant can determine the product characteristics. However, growers can maximize the number of maize cobs per plant to increase their income (Rinaldi *et al.*, 2023) and maintain animal feed sources.

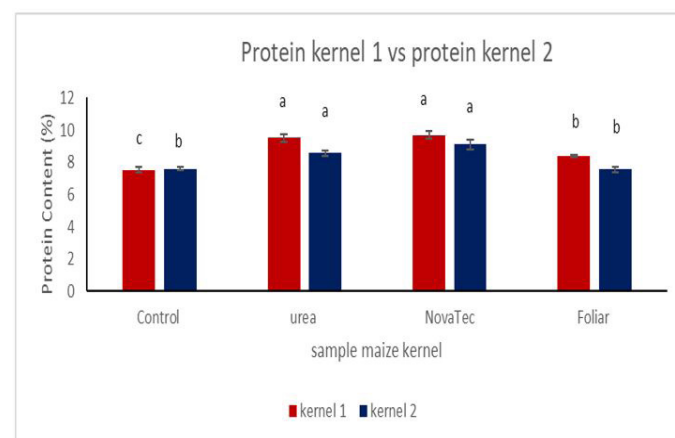


Figure 7: The protein content of different fertilizers of different maize kernels. Means followed by the same lowercase letters within the same colored column are significantly different according to tests LSD.

Apart from that, between leaf sections, the foliar fertilizers-treated maize has higher protein production, followed by NovaTec and urea (Figure 8). Since the foliar fertilizer was applied at the higher part of maize, the protein content in the leaf has increased instead of the kernel. The absorption of nitrogen nutrients in the leaf from foliar application is higher than that from basal application due to the nutrient uptake by stomata (Fernandez and Brown, 2013). Although the foliar was sprayed at the leaf area, the NovaTec fertilizers-treated leaf has slightly lower protein production for leaf specimens. According to Radulov *et al.* (2010), the N, P, and K-containing foliar fertilizers not only enhanced the protein content of maize but also affected the percentage of the various amino acids in maize's crude protein. Moreover, the maize leaf with two cobs showed higher protein production than the maize leaf with single cobs in foliar and NovaTec-treated leaf, while the opposite result was seen in control and urea. This situation may occur due to the nitrogen application decreasing the protein production in foliar and NovaTec fertilizers since the high nitrogen application can decrease the protein production. According to Stiegler *et al.* (2011), the high N application to the plant may reduce the protein content in the tissues. Thus, a sufficient amount of nitrogen applied to the plant can increase the protein content, while higher nitrogen application

can give the opposite results.

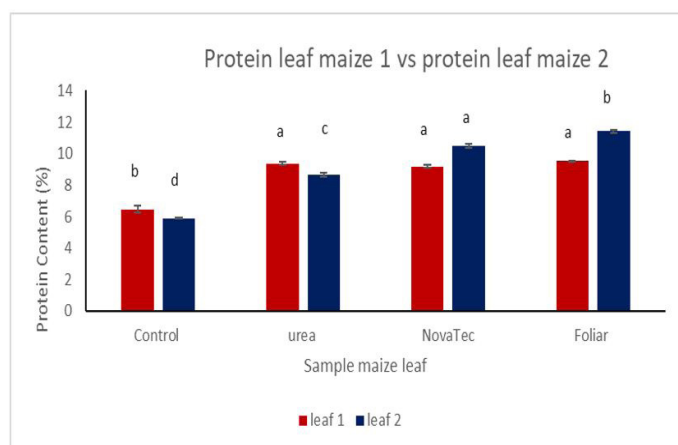


Figure 8: The protein content of different fertilizers of different maize leaves. Means followed by the same lowercase letters within the same colored column are significantly different according to tests LSD.

Other than that, the comparative analysis between kernel and leaf specimens showed that, generally, maize kernels have higher protein production than maize leaves (Figures 9 and 10). In Figure 9, kernel 1 has higher protein production in all treatments except foliar fertilizers-treated leaf 1. The result indicates that more crude protein can be produced in maize kernels than in maize leaves for single cobs. However, the result in Figure 10 showed different data, where leaf 2 had higher protein production than kernel 2, except in the control group. The protein production in the kernel was lower when there was an increased number of cobs per plant. The significant difference in foliar fertilizers-treated kernel and leaf may be due to the fertilizer influencing shoot growth but not root growth indices (Kentelky and Szekely-Varga, 2021).

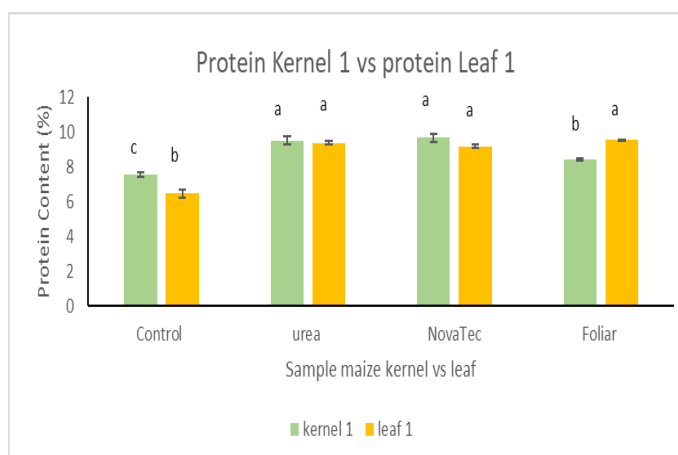


Figure 9: The protein content on different fertilizers of maize leaf 1 and kernel 1. Means followed by the same lowercase letters within the same colored column are significantly different according to tests LSD.

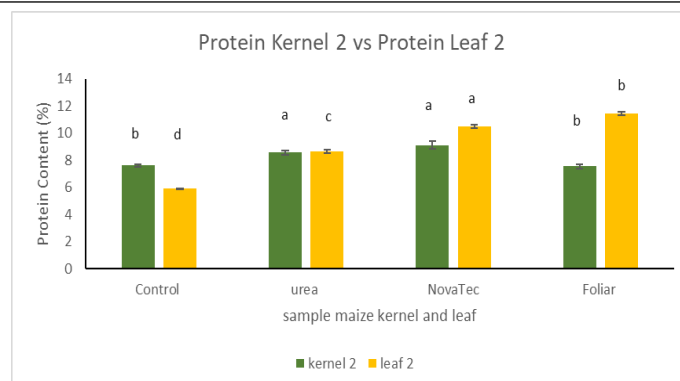


Figure 10: The protein content on different fertilizers of maize leaf 2 and kernel 2. Means followed by the same lowercase letters within the same colored column are significantly different according to tests LSD.

Previous studies also supported the differences in the crude protein contents between maize leaves and kernels. According to Ayasan *et al.* (2020), the highest crude protein content was within the leaves (12.41%), followed by the kernel (12.37%). Another study has reported that the crude protein content was higher in kernels compared to leaves by the application of fertilizer at the basal of maize (Wang *et al.*, 2023). Thus, it is suggested that crude protein production is mostly dependent on the amount of nitrogen given to the plant.

Conclusions and Recommendations

The present study has achieved the experimental objectives where there are differences in the morphological and physiological characteristics of the maize due to the difference in nitrogen application. Moreover, the different nitrogen applications have influenced the protein production in maize. The data from the present study has shown a significant difference in the crude protein production from different fertilizer treatments. The morphological characteristics, including the plant height, distance of nodes, chlorophyll content, and stomatal conductance, can increase with the increase in nitrogen applied. However, the leaf length and leaf width characteristics are similar between different nitrogen treatments. Furthermore, crude protein production is different for fertilizer treatments. The higher nitrogen content can induce higher production of crude protein in maize. Foliar fertilizers have a higher protein content in leaf kernel 1 and leaf kernel 2 than other fertilizers since they are applied to the leaf. This study can conclude that the NovaTec-treated fertilizer maize has the highest crude protein content compared to other fertilizer treatments.

Acknowledgments

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Novelty Statement

Nitrogen is a major nutrient needed by plants to produce protein. The crude protein produced is an important element in the animal diet. In the current study, the cultivation of maize with different nitrogen fertilizers was screened for protein production to determine the effect of nitrogen on the crude protein in different tissues.

Author's Contribution

Nur Amira Idayu Romzi, Aina Zahirah Mohd Suhaili and Mohd Fahmi Abu Bakar: Designed the study and analyzed the data.

Nur Amira Idayu Romzi, Aina Zahirah Mohd Suhaili and Norhayati Ngah: Performed the field work and collected the data.

Mohd Fahmi Abu Bakar and Norhayati Ngah: Drafted and reviewed the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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